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A Morphometric Analysis of the Highly Variable Clypeasteroid, *Periarchus lyelli*

Lauren Elizabeth Williamson
Wright State University

A MORPHOMETRIC ANALYSIS OF THE HIGHLY VARIABLE CLYPEASTEROID, *PERIARCHUS LYELLI*

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

By

LAUREN ELIZABETH WILLIAMSON
M.S.T., Wright State University, 2006
B.S., Texas A&M University, 2005

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Wright State University

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Lauren Elizabeth Williamson ENTITLED A Morphometric Analysis of the Highly Variable Clypeasteroid, *Periarchus lyelli* BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

Charles Ciampaglio, Ph.D.
Thesis Director

David Dominic, Ph.D.
Department Chair

Committee on
Final Examination

Charles Ciampaglio, Ph.D.
Thesis Director

David Dominic, Ph.D.

Burchard Carter, Ph.D.

Joseph F. Thomas, Jr., Ph.D.
Dean, School of Graduate Studies

ABSTRACT

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The Late Eocene echinoid, *Periarchus lyelli* (Conrad, 1834), known for its wide geographic range, high abundance, and specific stratigraphic range, is an ideal example of a guide fossil. However, due to its highly variable test morphology, many have questioned if, in fact, this sand dollar is actually two or three distinct species that have been misclassified.

A preliminary study on this subject has been performed on specimens from Mississippi, North Carolina and South Carolina (Williamson, 2006), showing significant separation in test shapes. Continuing previous research, this study analyzes the test shapes of *P. lyelli* over its entire North American geographic distribution.

Multivariate statistical techniques combined with substrate and structural analysis support the idea of three species instead of one in the case of *P. lyelli*. This examination of *P. lyelli* provides insight to the precise classification of the sand dollar and the difference between interspecies variation and intraspecies variation.

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1.0 INTRODUCTION

In order to present an unbiased study of the morphology of *Periarchus lyelli*, a quantitative approach must be used. By observation alone, many differences in profile shape can be seen among *P. lyelli* specimens. While simple physical measurements such as test margin thickness or peristome/periproct position can be used to show differences, the only way to remove as much bias as possible from these studies is to perform multivariate statistical analyses. A study utilizing shape analysis can determine the relationships among specimens. It is possible that environmental differences related to geographic distribution may play a part in the differing shapes, or ecophenotypic variations. Over time, ecophenotypic variation can lead to reproductive isolation, and eventually speciation (Prothero, 2003).

One such environmental factor could be sediment type. It is possible that some populations of *Periarchus* prefer carbonate substrates and that some prefer detrital substrates. If these populations differ in morphology, it would indicate a correlation between profile shape and sediment type. Sediment type, as referenced in this study is not to be confused with geologic unit or formation, as units can change with geography and age. Age may play a part, as some populations could be from younger rocks and some from older ones.

Changes in environmental stimuli could provide the basis for an adaptation that led to a change in the structure of *P. lyelli*. These factors could include climate, oxygen saturation, or other environmental factors that may have affected the structure in some way. A structural or internal architectural difference from other populations of *Periarchus* would warrant separation of the species. Previous publications have suggested that this species should be separated based on other evidence such as external morphologic characters (Ravenel, 1844, Paulson, 1958, Fischer, 1951; Kier, 1980;).

For the purposes of this study, several methods were employed, including multivariate statistical analysis, sedimentological techniques, and an internal analysis of the structure of the sand dollar. After the morphology was examined, Geographic Information Systems (GIS) was used to see if there is any connection between morphology and geography. Taken together, the results of these studies indicate that the current classification of *P. lyelli* is erroneous.

The goals of this project are to determine: (1)what, if any, physical environmental factors are correlated with test shapes in different localities; (2) if this trend continued throughout the North American distribution of *P. lyelli*; and (3) tif these differences are separated morphometrically and are significant enough to be considered a species difference.

2.0 BACKGROUND

2.1 Biology

Periarchus lyelli is generally considered an irregular echinoid, but is officially classified as follows:

Kingdom Animalia

Phylum Echinodermata

Class Echinoidea

Superorder Gnathostomata

Order Clypeasteroidea

Suborder Scutellina

Family Protoscutellidae

Genus *Periarchus*

Species *lyelli* (Moore, 1966)

Most irregular forms retain Aristotle's lantern, or the chewing mechanism for echinoids, though the purpose of the structure is more supportive, unlike that in regular echinoids (Lawrence, 1987). Clypeasteroids inhabit a variety of sediments, however, *P. lyelli*, mainly lives in shallow, relatively coarse substrata, or coarse lime sediment in high-energy marine environments (Prothero, 2003).

The relatively large test of *P. lyelli* increases the surface area on the oral surface and allows for increased food collection, while the thin outer margin is ideal for burrowing. The upper surface serves as a sieving mechanism for food collection.

Particles drop between spines onto the test surface where cilia move them to the margin for transfer to the oral surface. A thin test margin could also aid in the transfer of food from the aboral to the oral surface (Lawrence, 1987) (Fig. 1).

In many echinoderms, the apical cone is the part of the aboral plate that includes the ambulacra, and rises into a dome with the apex at the center. This structure is used by individuals to keep a connection to the water column when buried (Lawrence, 1987). Some specimens of *P. lyelli* have a higher apex than others, suggesting this function of the anatomical feature.

Irregular echinoids such as *P. lyelli* also display two types of symmetry. Pentameral symmetry is readily seen in the five ambulacral petals on the aboral surface and sets of food grooves on the oral surface. A plane of symmetry passes through each petal, meeting at the apex. A plane of symmetry passes through the middle of the upper ambulacrum and between the two lower ambulacra on the aboral surface, and through the peristome and the periproct on the oral surface (Fig. 2). Bilateral symmetry is a characteristic that aids this type of echinoid in unidirectional movement within sediment. This can be seen by looking at the aboral surface with the ventral side closest to the observer. In addition to this, some specimens of *P. lyelli* are slightly elongated (Cooke,1959; Clark and Twitchell, 1915; Kier, 1980). Most sand dollars locomote in the direction of elongation, which allows for easier burrowing and suggests increased rate of burrowing. (Lawrence, 1987; Cooke, 1959; Kier, 1980).

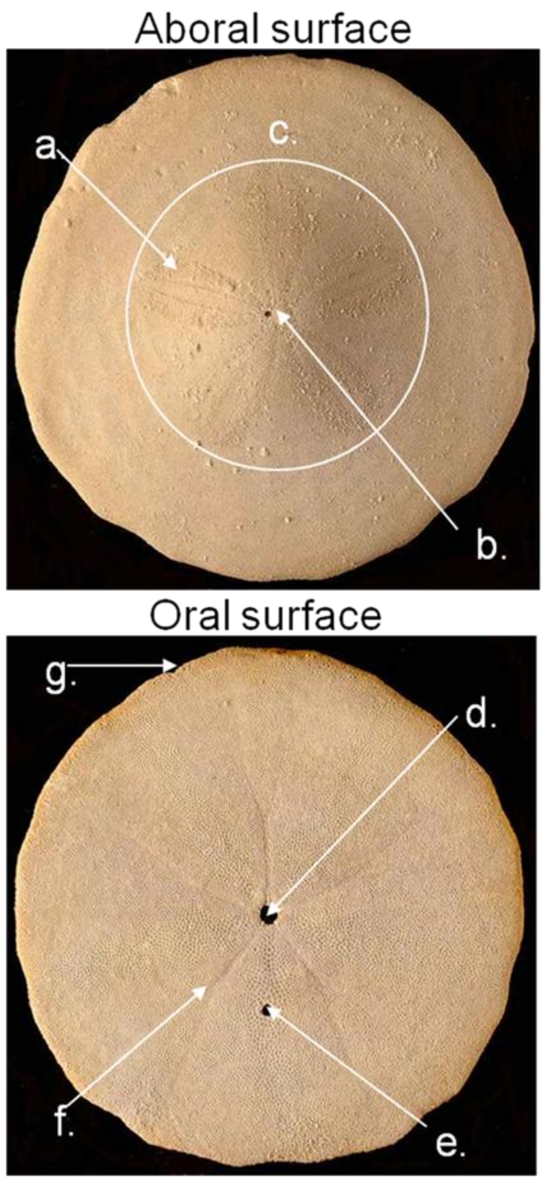


Figure 1: Diagram of *P. lyelli* morphology. a. ambulacrum, b. apex, c. apical cone, d. peristome, e. periproct, f. food groove, g. test margin. Photographs from Smith, 2005.

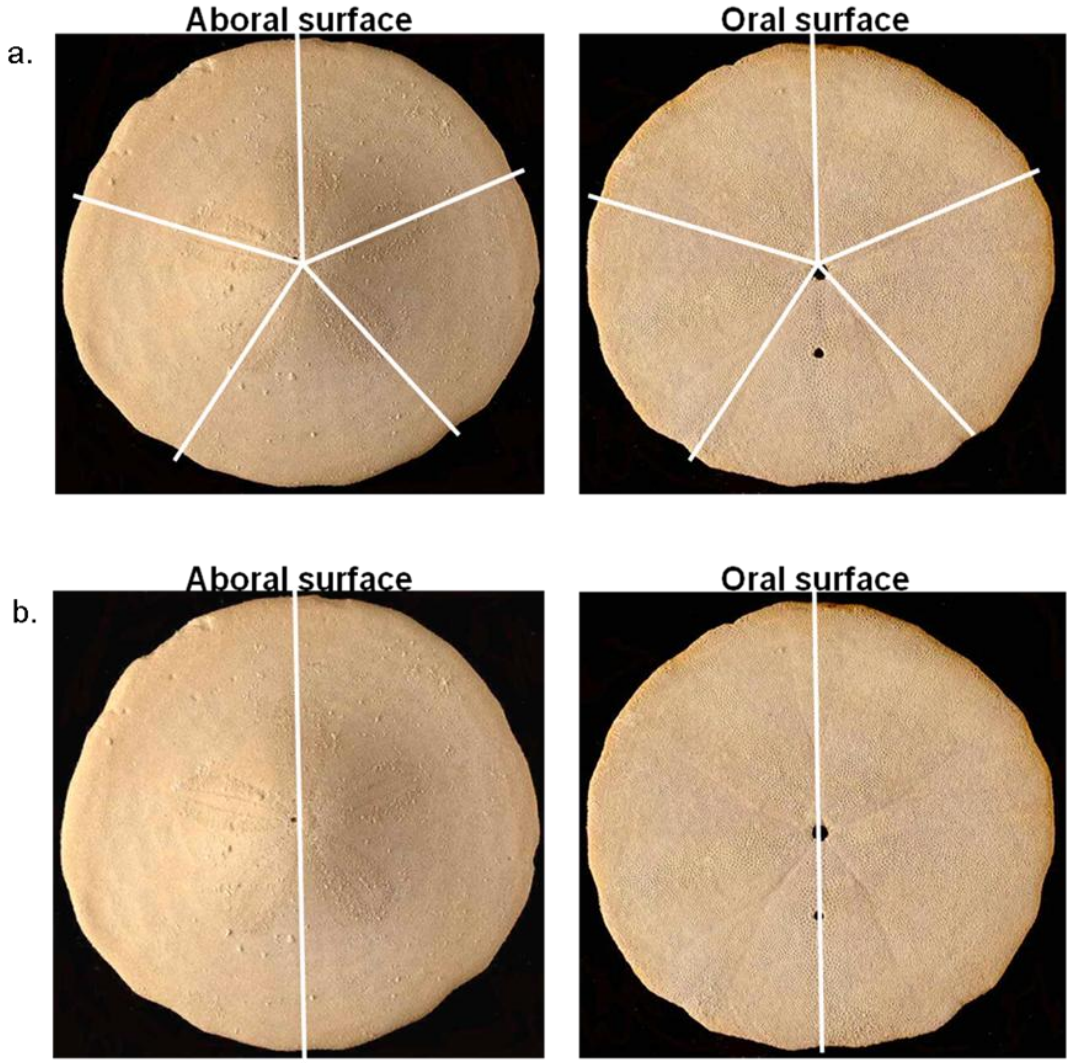


Figure 2: Diagram of *P. lyelli* symmetry. a. planes of pentameral symmetry, b. plane of bilateral symmetry. Photographs from Smith, 2005.

2.2 Published Works

The Clypeasteroid *P. lyelli* was originally described by Conrad (1834). The holotype was described as having a subcircular test with the periproct positioned 1/2 to 1/3 of the way between the peristome and the outer margin. Others have described this echinoid as having a subrounded cross-sectional shape with some profiles sharply convex and others gently domed (Kier, 1980), or as a gently rounded mound that was sometimes conical (Clark and Twitchell, 1915). It is agreed by all workers that the morphology of *P. lyelli* is highly variable.

The geographic range extends along the Gulf coast of North America and includes part of the southern Atlantic coast of North America as well. Units containing this echinoid include the Moodys Branch Formation, Cook Mountain Formation, Ocala Limestone, Santee Limestone, and Castle Hayne Limestone (Conrad, 1834; Clark and Twitchell, 1915; Cooke, 1959; Kier, 1980; Carter, 1987; ; Zachos and Molineux, 2003) (Fig. 3). Over the last 150 years, many workers have split *P. lyelli* into several species or subspecies based on observational interpretation of morphology. The species *P. rutriformus* (Paulson, 1958) was named, as well as subspecies *P. lyelli pileussinensis* (Ravenel, 1844), and *P. lyelli floridanus* (Fischer, 1951) (Fig. 4). *P. rutriformis* was said by Paulson (1958) to have a smaller size, and several differences in the distribution of food grooves and basicoronal plates. *P. lyelli pileussinensis* was named for the pointed shape of its profile, resembling a Chinese hat, and was found mainly in Mississippi and Alabama. *P. lyelli floridanus* was described as having a very flat profile compared to the slight dome in *P. lyelli*, and was found mainly in Florida, as the name suggests.

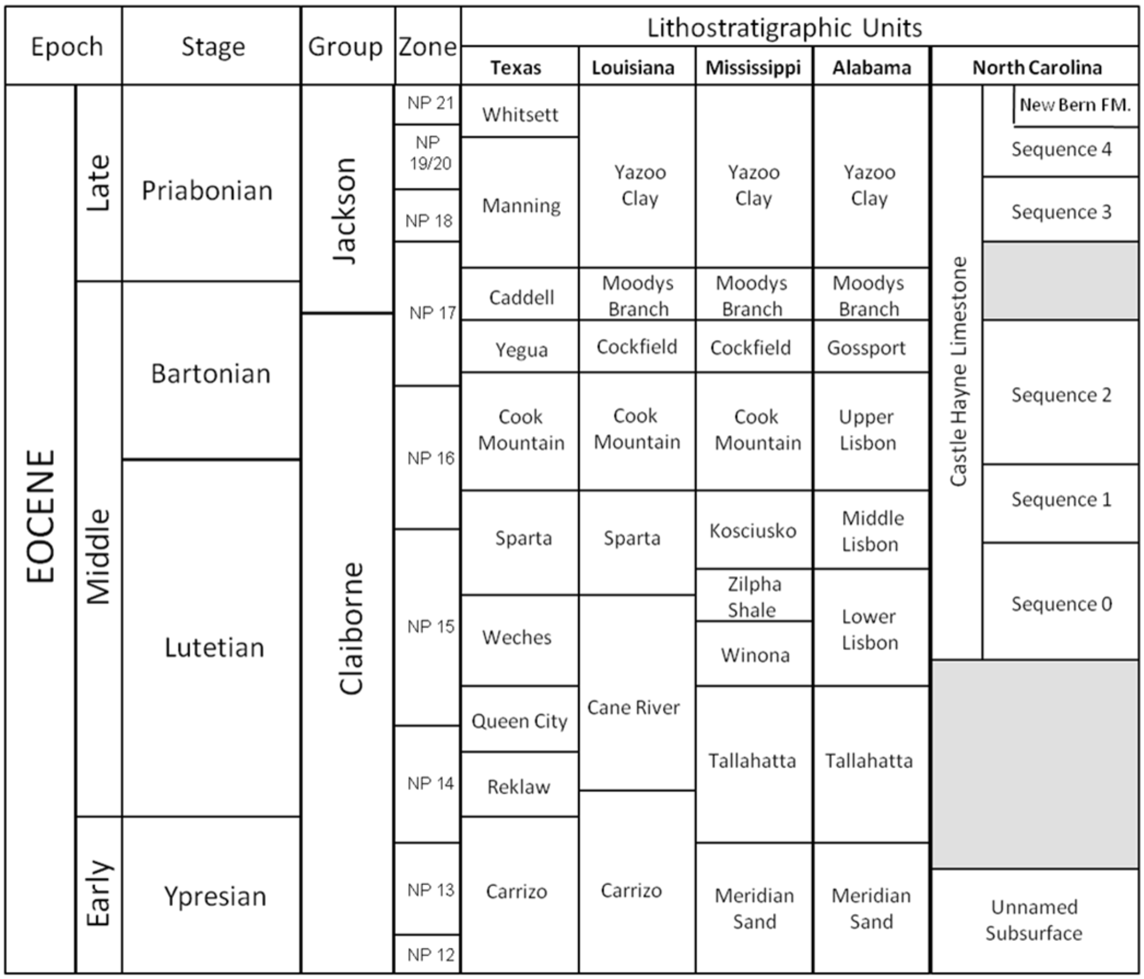


Figure 3: Eocene age units of formations in the Gulf coast and southern Atlantic coast of North America. Courtesy of Dr. Chuck Ciampaglio.

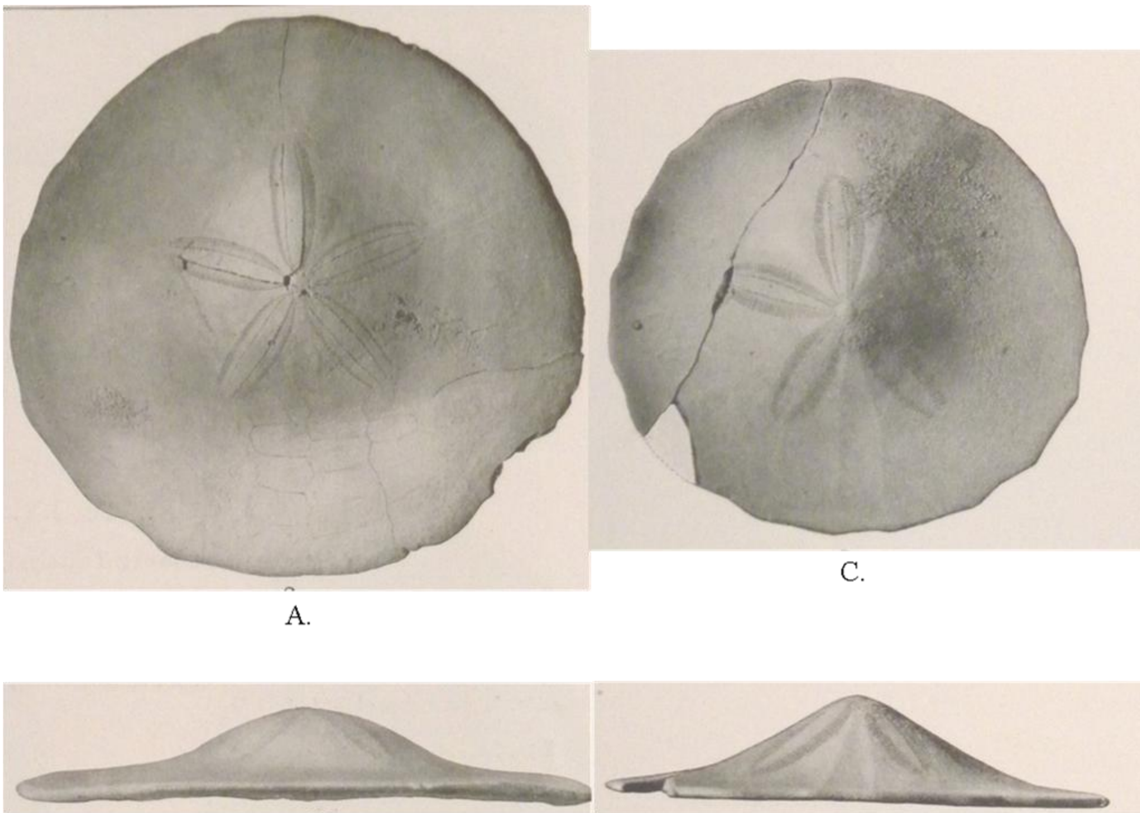


Figure 4: Examples of *Periarchus* specimens; *Periarchus lyelli*: A. Top view, B. Profile view; *Periarchus lyelli pileussinensis*: C. Top view, D. Profile view. Pictures from Clark and Twitchell, 1915.

However, the traditional *P. lyelli* has been found over the entire span of the geographic range.

Kier (1980) synonomized *P. rutriformis* into *P. lyelli*, stating that all specimens are essentially *P. lyelli* because the evidence for separation of the species was unfounded and biased, and the form is most likely a smaller or more juvenile version of *P. lyelli*. There is much debate about the species status of *P. lyelli pileus-sinensus* and *floridanus* since they are so similar to the basic form of *P. lyelli*. In the years since, no work has been performed to formalize the separation of these species further than the subspecies level. Therefore, even though many continue to use this old classification, all are still technically *P. lyelli*.

2.3 Previous Study

A preliminary analysis (Williamson, 2006) was performed to determine if a full morphometric analysis was warranted. Several studies were included in this project, including a sensitivity analysis and Elliptical Fourier Analysis (EFA) combined with Principal Component Analysis (PCA) on specimens from a specific cross-section of the geographic distribution of the species. *P. lyelli* specimens were collected from the Moody's Branch Formation in Mississippi, the Santee Formation in South Carolina and the Castle Hayne Formation in North Carolina. All were analyzed with EFA and PCA. Results from the PCA clustering were then reformatted in a spreadsheet, and samples were separated by unit on a cluster graph. Based on distinct clusters within the plot, Williamson, 2006 concluded that specimens from the Moodys Branch in Mississippi are clearly different from those collected from the Santee in South Carolina and the Castle Hayne in North Carolina (Fig. 5).

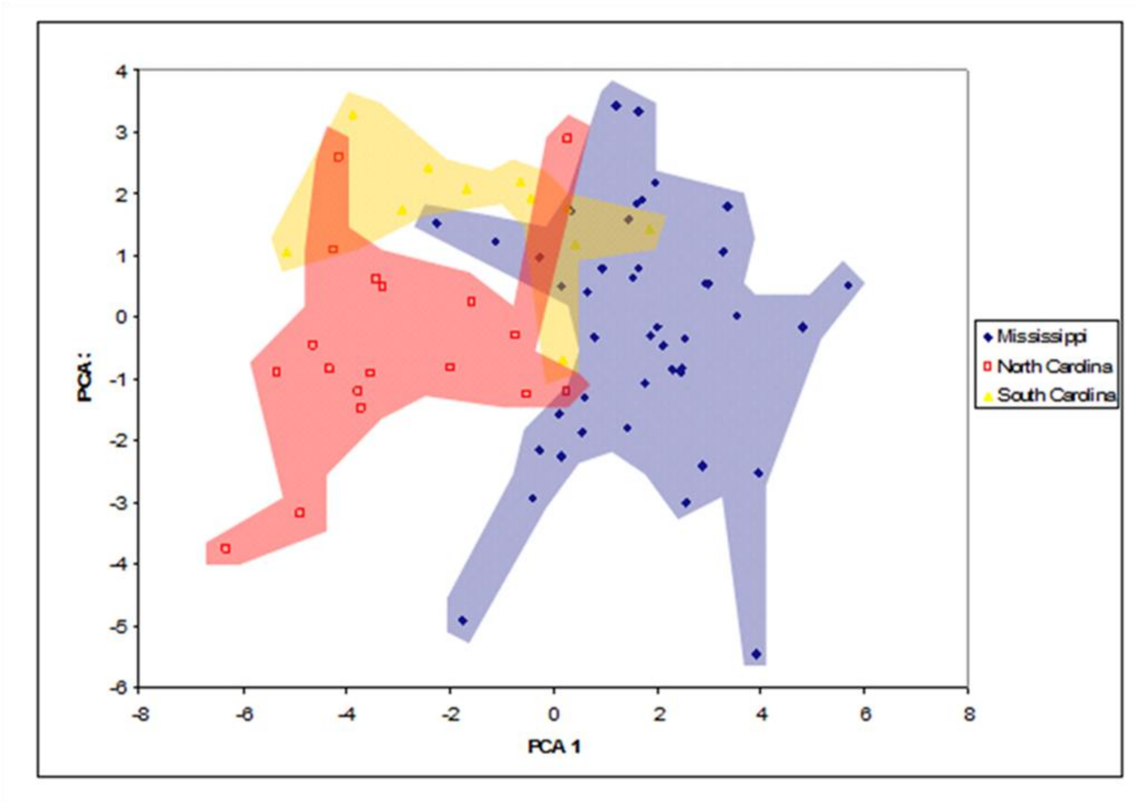


Figure 5
Graph of PCA 1 and 3 showing the relationships between all *P. lyelli* specimens used in the preliminary study

2.4 Blastoid Study

In addition to the techniques used in the previous study of *P. lyelli*, I wished to test the effectiveness of Procrustes Analysis and subsequent PCA. Therefore, a study was performed upon several taxa of another type of echinoderm. Blastoids are members of the same Phylum as echinoids, and share certain traits with *P. lyelli*. For instance, the two organisms have high magnesium calcium carbonate stereom plates that make up their tests, and pentameral symmetry associated with the petal-like ambulacra (Prothero, 2003) (Fig. 6).

The purpose of the sensitivity analysis was to determine if a morphometric analysis upon three separate but related blastoid taxa would result in three distinct clusters. Upon first inspection, specimens of *Tricoelocrinus woodmani* and specimens of *Metablastus wortheni* look to be more similar in morphology than *M. wortheni* and other species of *Metablastus.* A quantitative study of morphology using multivariate statistical analysis showed definitively which taxa are more closely related.

Elliptical Fourier Analysis (EFA) and Procrustes Landmark Analysis were used in conjunction with Principal Component Analysis (PCA) to create cluster graphs. Additionally, a comparison was made between length of theca, or calyx, and the variable representing the largest amount of variation in the sample set. PCA 1 represents this variable, since it captures the largest amount of variation within the sample set.
The results of the EFA and PCA show a clear clustering between *T. woodmani* and *M. wortheni* and a clear separation of this cluster from other *Metablastus* species (Fig. 7). These results are echoed in the Procrustes results (Fig. 8). The Length vs. PCA1 graph reinforces these findings, showing a linear trend involving *T. woodmani* and *M.wortheni.*

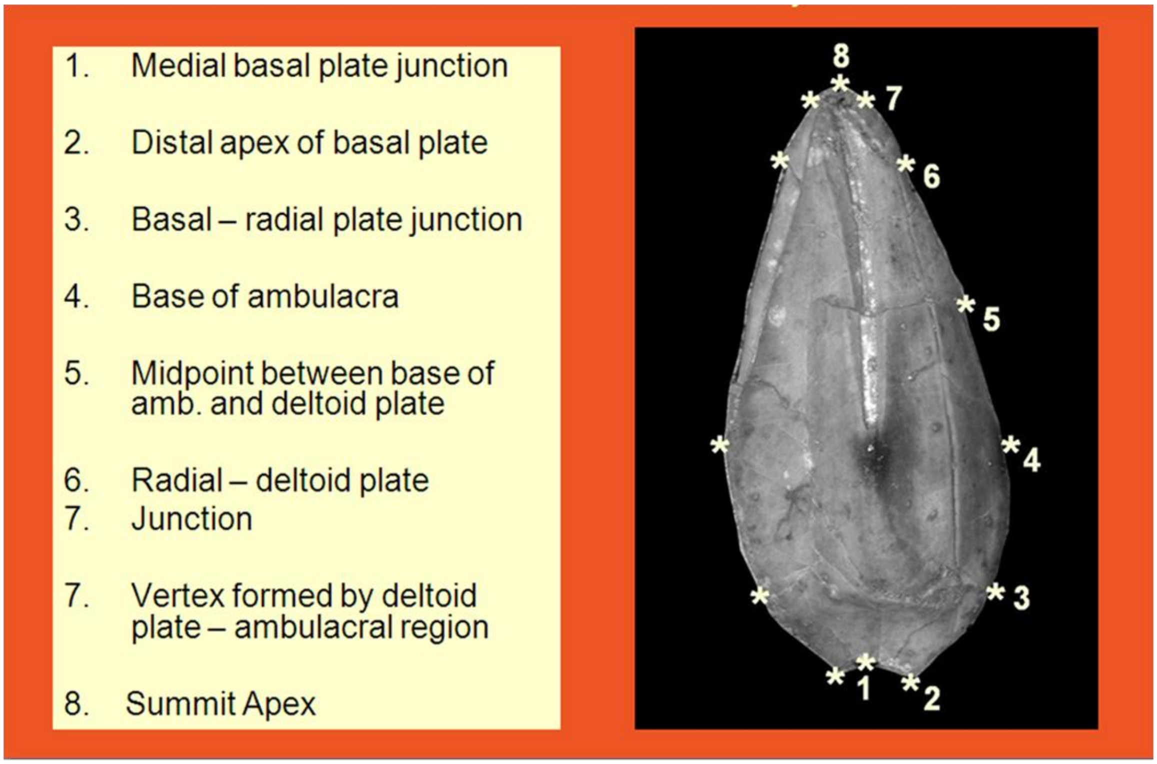


Figure 6: Diagram showing example landmark positions for Blastoid Procrustes analysis.

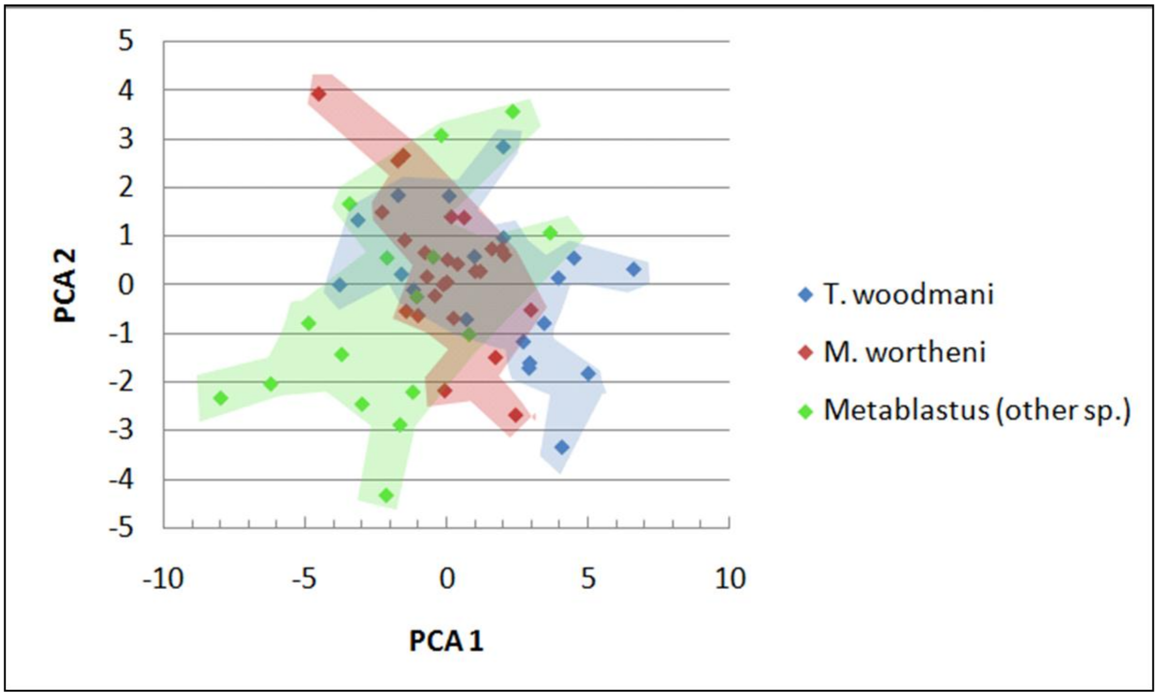


Figure 7: Scatterplot of Principal Component Axes 1 and 2 in Blastoid EFA.

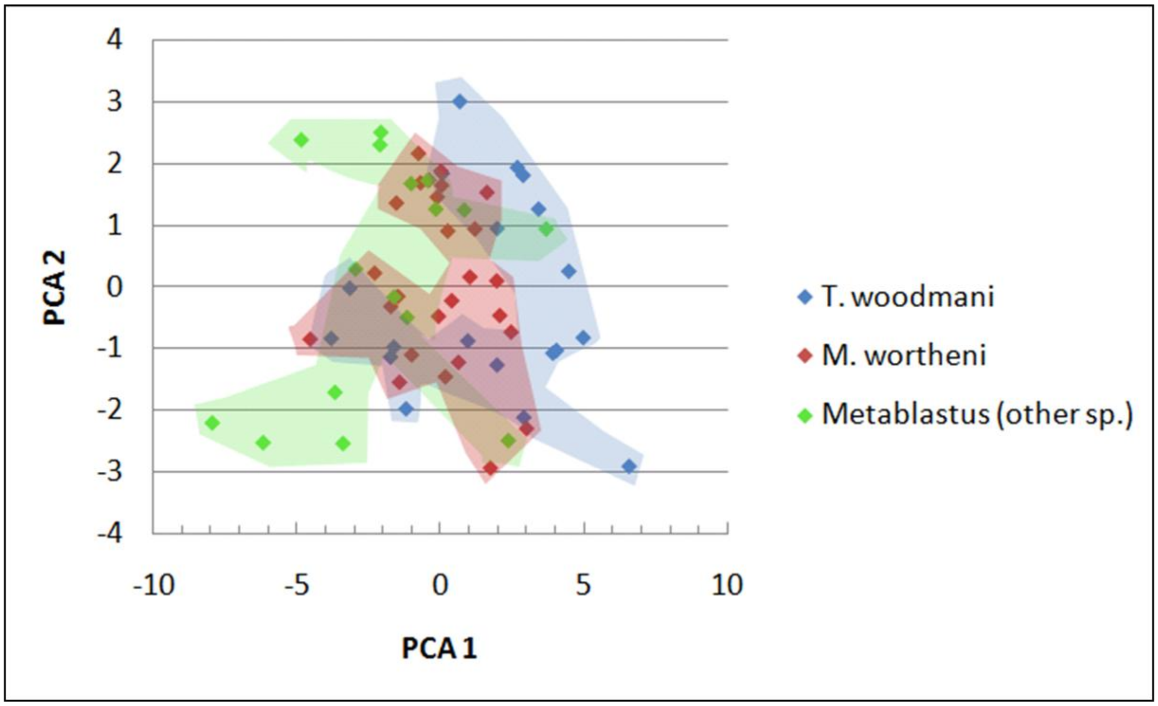


Figure 8: Scatterplot of Principal Component Axes 1 and 2 in Blastoid Procrustes analysis.

Metablastus species other than *M. wortheni* plot in a cluster scattered around the graph, not linearly like the other two (Fig. 9). These results show not only that *T. woodmani* and *M. wortheni* are more closely related than their classification suggests, but that a reclassification is necessary, reassigning the species *T. woodmani* to the genus *Metablastus*. This sensitivity analysis also shows that EFA and Procrustes, paired with PCA and other statistical techniques give meaningful, quantitative results.

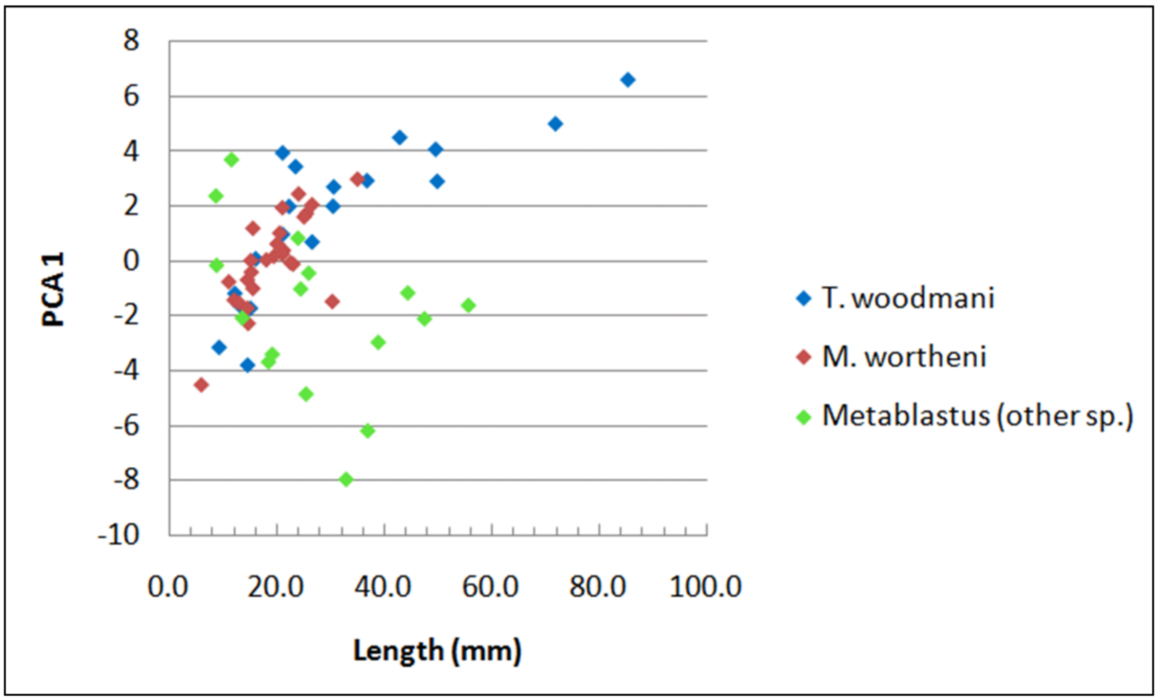


Figure 9: Graph comparing length of Blastoid theca and Principal Component Axis 1 of all Blastoids (data taken from Procrustes analysis).

3.0 METHODOLOGY

3.1 Collection and Preparation

Specimens of *Periarchus* were collected across the entire geographic range for the genus. The units included in this range are found in the states bordering the Gulf coast and southern Atlantic coast of North America. The specimens from Mississippi (MS 1-43), North Carolina (NC 1-21), South Carolina (SC 1-19), and Georgia (GA 1-31) were collected specifically for this study. Specimens were collected in situ and were taken back to the lab for preparation and cataloging (Fig. 10, 11 and 12; Table 1). Each specimen was cleaned of all remaining sediment and specific morphological characters were measured and checked for classification purposes. The specimens were washed clean, and any broken pieces or cracks were stabilized with Paleobond®. The clean specimens were then photographed from the top (aboral surface) and in lateral profile. All specimens were oriented in lateral profile with ambulacrum III to the right, perpendicular to the plane of bilateral symmetry.

Periarchus from localities in Texas (TX 1-7) and Louisiana (LA 1) were from the catalogued collections of the Texas Memorial Museum at the University of Texas at Austin. Photographs of these specimens were used with permission for this study. *Periarchus* specimens from localities in Alabama (AL 1-25) were from the catalogued collections of the Geological Survey of Alabama, and photographs of the specimens were used with permission for this study.

Figure 10: Photo of collection site at Castle Hayne Quarry, North Carolina.

Figure 11
Photo of collection site at La Farge Quarry, South Carolina

Figure 12: Photo of *P. lyelli* specimen preparation.

State	Collection Locality	Stratigraphic Unit	Geologic Description	Other Fossils
Mississippi	Outcrops along the Chickasawhay River, Shubuta Creek, and Techeva Creek, Mississippi	Moodys Branch Formation	Blue/gray, clayey quartz sand with shells embedded in glauconite, sandy limestone beds	Gastropods, Pelecypods
North Carolina	Castle Hayne Quarry along the east coast in Wilmington, North Carolina	Castle Hayne Formation	Light tan to white Limestone	Gastropods, Pelecypods, Bryozoans, Forams, Diatoms, Regular and Irregular Echinoids
South Carolina	La Farge Quarry, South Carolina	Santee Formation, Cross Member	Calcarenite Somewhat sandy Limestone	Pelecypods, Bryozoans
Alabama	Conecuh River at points 4 and 6 miles south of Andalusia, Alabama; Alabama River, Left Bank at mile 69.8 (Rattlesnake Bluff); Double Bridges Creek; Chattahoochie River, at mile 36.4	Moodys Branch and Gosport Formations	Tan fine to medium-grained, significantly porous, fossiliferous sandy limestone Glauconitic, clayey marl	Gastropods, Pelecypods, Forams and Echinoids
Florida	Cross State Canal; Big Periarchus Pit, Levy County; Green Sink and Marion Northside Stone, Marion County; Dolime Quarry, Citrus County	Ocala Formation	Calcarenite/micrite White to cream porous limestone with occasional dolostones. Composition is almost pure $CaCO_3$, no quartz	Gastropods, Pelecypods
Georgia	Quarry in Perry, Georgia; south of Macon, central Georgia	Tivola Formation (Ocala Group)	Calcarenite/micrite White to cream porous limestone with occasional dolostones. Composition is almost pure $CaCO_3$	Gastropods, Pelecypods, Bryozoans, Forams
Texas	Sabine County, along the eastern border of Texas	Cook Mountain Formation, Caddell Formation	Marl, limestone, and glauconitic sand	Gastropods, Pelecypods, and Echinoids
Louisiana	Left bank of the Red River, about 1 mile southwest of Montgomery, Louisiana	Moodys Branch Formation	Tan fine to medium-grained, significantly porous, fossiliferous sandy limestone	Gastropods, Pelecypods

Table 1: Descriptions of collection localities by state

Periarchus specimens from Florida are a part of the collection at the Florida Museum of Natural History. FL 1-6 (intact tests of *P. lyelli*), and FL 7-12 (external molds of the aboral surface and corresponding silicone peels), were borrowed and used with permission for this study.

All photos were then edited in Photoshop® and converted to appropriate formats for Procrustes analysis and EFA. For EFA, the images were converted to black and white bitmap files to minimize the noise and bring focus to the outline shape. For Procrustes, the pictures were simply sharpened for clarity, so that significant morphologic features could be easily analyzed.

In addition to photographs, physical measurements were also taken of each specimen. Features measured include apical height, thickness of test margin, length of ambulacra, distance between peristome and edge of test and distance between periproct and edge of test. All measurements were taken in millimeters.

3.2 Elliptical Fourier Analysis

The edited image files were analyzed with the morphometrics program SHAPE (Iwata and Ukai, 2002). The SHAPE program consists of several applications that are packaged together in order to perform an Elliptical Fourier Analysis. Procedurally, the first step is to convert bitmap images into a file that contains geometrical information about contours called chain code. The resulting chain code is translated into a normalized Elliptic Fourier expression consisting of a desired number of harmonics. Nine harmonics are able to capture the basic outline with maximal detail and little “noise” or unnecessary information. Since the sand dollar shapes are relatively uncomplicated curves, there was no reason to use more harmonics. This method is explained in further detail in Appendix A.

Each harmonic is described by four coefficients that are saved in the final normalized Elliptic Fourier data file (Fig. 13). Each data file represents one specimen, and forms a 1 x 36 row vector. Each row vector is inserted into a spreadsheet form in an *m* x 36 data matrix (where *m* = number of specimens). A correlation matrix analysis was then performed using the multivariate statistical program, PC-ORD (McCune and Mefford, 1999). The resulting data were graphed and analyzed for evidence of discrete shape clusters.

3.3 Procrustes Analysis

For the Procrustes analysis, photographs were taken with enough resolution to be able to see the detail of the profile of the test. The photographs were edited for clarity and sharpness, and the specimens were examined for features, such as ambulacral petals, that are common and could be easily seen in every photo. In Procrustes analyses, landmarks are chosen at common points throughout the sample set. For example, in the Procrustes analysis of the Blastoids *T. woodmani* and *Metablastus sp*., landmarks were chosen according to the structure of the theca. Specifically, landmarks were placed at the suture points between plates. In *Periarchus*, suture points between plates are very difficult to see in photos of this scale in profile. Therefore, landmarks were chosen according to key morphologic characteristics common to every specimen.

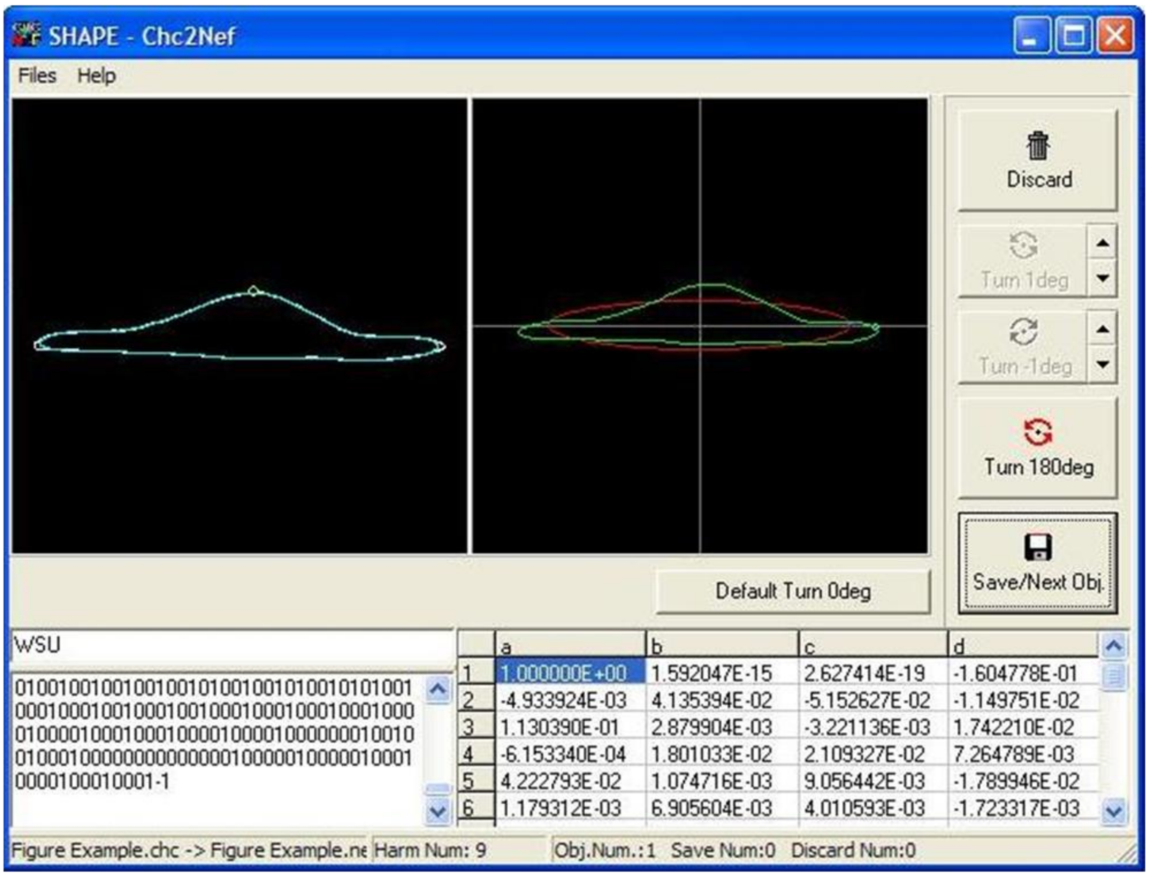


Figure 13: Screenshot of the SHAPE program performing Elliptical Fourier Analysis on specimen MS 1. This shows shape capture (Top Left), normalization (Top Right), chain code (Bottom Left), and the resulting Fourier coefficients needed for further analysis with PCA (Bottom Right).

A total of 14 landmarks were chosen on each specimen (Fig. 14). Landmarks 1, 2, 3 and 4 define the thickness of the outer margin of the test. Landmark 1 is at the bottom edge of the side of the profile near the peristome (the left side of the photograph of the profile), and landmark 2 is positioned at the top of the test margin on the same side. Landmark 3 is similarly located at the top of the outer margin on the right side of the profile, and landmark 4 is at the bottom of the test directly below landmark 3. Landmarks 5, 6 and 7 locate the ends of the ambulacra that can be seen in profile. Landmark 8 on the left side of the test as viewed in the photograph, and landmark 14 on the right side of the test as viewed in the photograph locate the inversion point on the aboral surface where the test begins to incline toward the apex. Landmark 9 on the left side of the test, and landmark 13 on the right side of the test locate the inversion point on the aboral surface where the test either steepens its incline toward the apex, or becomes convex toward the apex. Landmarks 10, 11 and 12 define the apex of the sand dollar. Landmarks 10 and 11 are the ends of ambulacra II and III on the right and left sides of the apex, and landmark 12 is the apex itself.

These landmarks were then measured with the program Image J (Rasband, 2008), and x and y coordinates were assigned for each. These values were imported to a spreadsheet so that all coordinates were included in one row vector per specimen. The data file was uploaded into the program PAST (Ferson, 1985), and the coordinates were transformed into Procrustes coordinates. The resulting data were then analyzed with the PCA tool included in PAST and scatterplots were produced using Principal Component Axes 1, 2 and 3. All PCA results were analyzed in a similar fashion to that from the Elliptical Fourier Analysis, focusing on finding distinct clusters in the plots.

Figure 14: Screenshot showing placement of landmark coordinates on specimen AL1 using the program Image J. Specimen is positioned with Ambulacrum II toward viewer, and Ambulacrum III to the right.

3.4 Sediment Analysis

In the course of collection and preparation, samples of the substrate were taken from each location. Many *P. lyelli* specimens were very delicate, and therefore, blocks of the unit had to be taken to make sure the sand dollar remained intact. When those specimens were being prepared and cleaned, pieces of the surrounding rock were saved for analysis. Descriptions of the units were then made from notes in the field and from this residual matrix. Color, grain size, composition, and other fossils were carefully noted for each collection locality. From this information and the geologic history of the area, the paleoenvironment of each locality can be inferred.

For two key localities, Perry, Georgia and Rattlesnake Bluff, Alabama, a closer analysis was performed in order to compare the sediment. A small block of each was dried in an oven to remove any excess water from within the pores. Then, the blocks were soaked in epoxy to impregnate the samples. After the epoxy hardened, the blocks were sanded with increasingly fine sandpaper to remove any tool marks and provide a smooth, polished surface. A thin section was prepared from each of the finished blocks. Once prepared, the slides were etched using 1.5% HCl and stained with Potassium Ferricyanide and Alizarin Red S (ARS/PF) in order to make the calcium carbonate stand out under the microscope and to check for the presence of iron in the sediment.

3.5 Geographic Information Systems (GIS)

In addition to the physical and quantitative studies of this echinoid genus, the locality information was analyzed in a geographic perspective. The objective of the GIS study in this project was to analyze the geographic distribution of *P. lyelli*, to show the

Eocene geologic units in the selected area, to connect information from each collection site to the map, and to look at the distribution of the genus according to several different components such as geologic unit, sediment type, and geography.

First, a map of the continental United States was downloaded from the USGS website. All information included with the downloaded data, including the coordinate system, was added to the initial data frame. These data were combined with another map of the contiguous United States, transforming the projection where necessary. The result was a map of the United States with each individual state outlined, overlain by the geologic units.

The next step was to isolate the data necessary for this specific study. Only the units of Eocene age were needed, therefore they were selected. A layer was created for all of the Eocene units collectively, and individual layers for each individual Eocene unit were also created. For this task, the *Select by Attributes* function was used for each unit, and then a layer was created from each of the selected features. The geographic area was then isolated by selecting the states sampled individually, and creating layers for each of them using the same method as was used for the geologic units. To create a continuous section, these state layers were then appended to one another using *ArcToolbox* and the *Data Management Tools*. This resulted in a layer that included only the states between Texas and North Carolina along the Gulf coast and Southern Atlantic coast of the United States. Then, *ArcToolbox* was again used, with the *Analysis Extract* tool helping to clip the map features. The outlines of the states were clipped to only include such sampled states, and the Eocene geologic units were clipped to only include parts inside the sampled states.

Next, a new Data Frame was inserted, and the state outline layer, clipped states layer, and clipped Eocene layer were added to the Data Frame. Each geologic unit was then selected individually using the *Select by Attributes* option, and layers were created of each, utilizing the same method as before. The next step in the process was to add a layer showing the sample collection sites across the geographic area. First, the layers showing cities, rivers, interstates, and major roads were added to help identify the correct features surrounding the sample sites. Next, the shapefile, P.lyelli_sites, was created in *Arc Catalog*, and edited using *ArcEditor*. Points were placed carefully, marking the location of each specific collection site. An Excel® file was added to the Data Frame that included detailed information about each sample collection site. To connect this data with the new shapefile, *ArcToolbox* was used to create a table from the Excel file using the *Conversion Tools*. The resulting table was joined with the attribute table of the sample collection site layer. Finally, in order to make the data easier to access and interpret, the two data frames were then organized in layout view with a legend and scale (Fig. 15).

3.6 Structural Analysis

For the structural analysis, several specimens were chosen from the sample set, representing all observed profile shapes as defined by morphotype, localities, and sediment types. Specimens chosen were SC8, GA14, SC 13, NC 18, and MS 22. Each specimen was soaked in Paleobond® and allowed to dry. After the Paleobond® was no longer tacky, the specimens were placed in an oven for a few minutes to ensure dryness. When completely prepared, the specimens

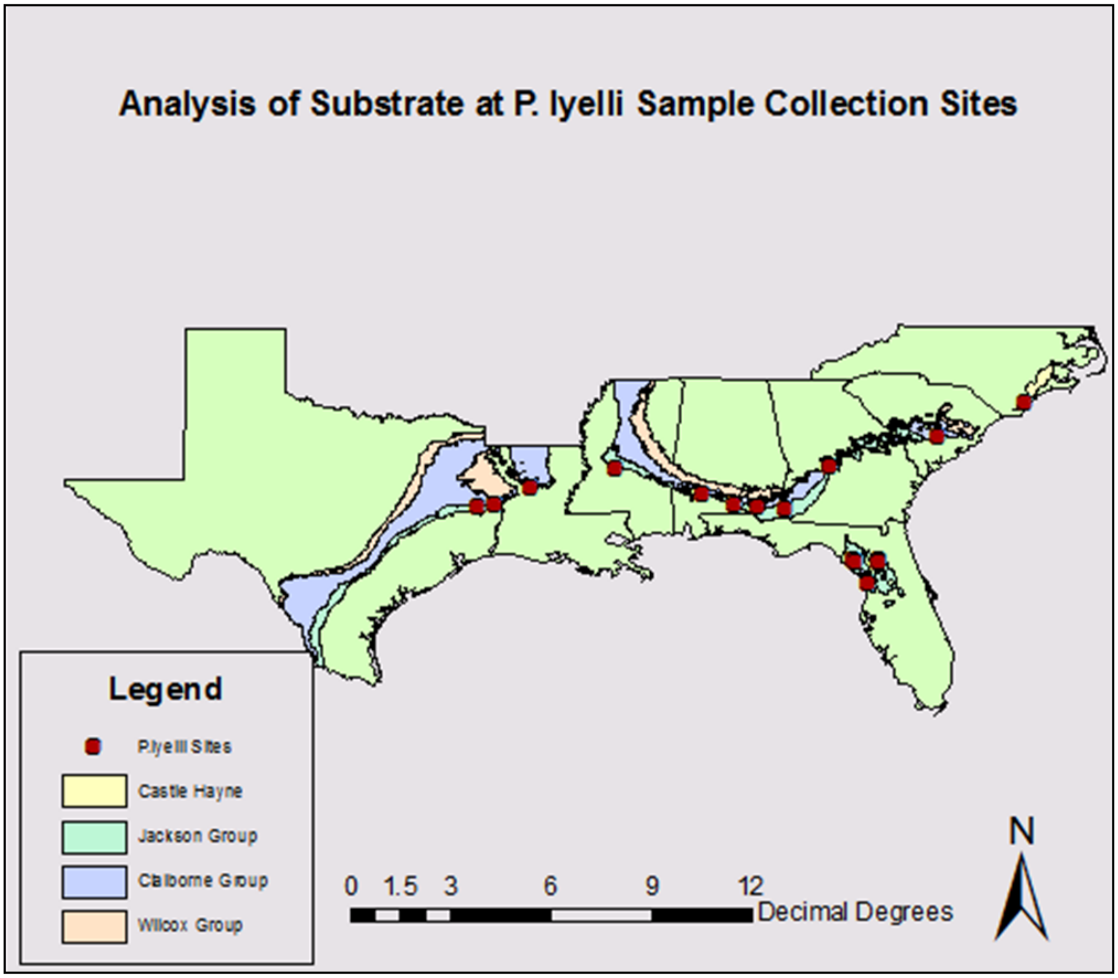


Figure 15: Map of collection localities and Eocene-age geologic units.

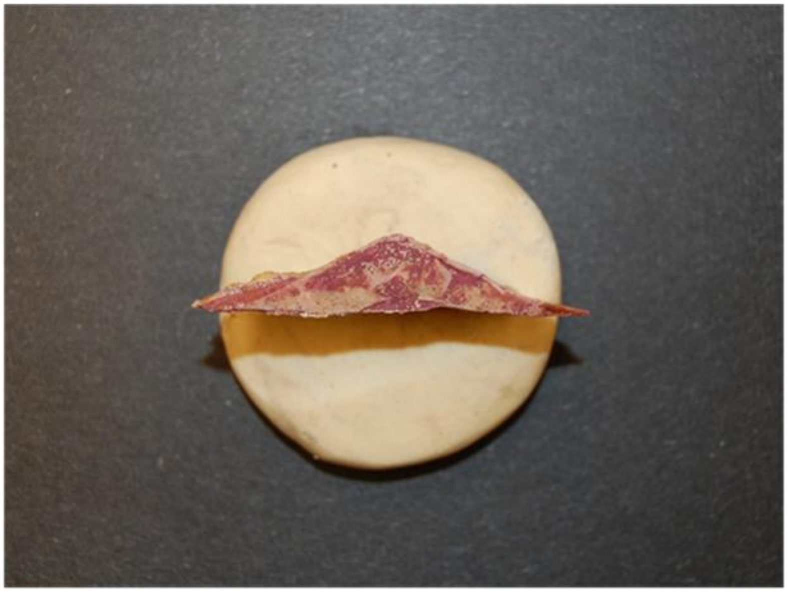
were sliced with a rock saw along the plane of bilateral symmetry through the peristome and periproct. The sliced samples were then sanded to polish the newly exposed faces, and examined under a microscope. In addition to examining the supports from the profile perspective, one side of the sand dollar was sanded down around the apex so that the interior could be seen from above.

The sediment on the inside of the test made it difficult to distinguish the interior support structures. Therefore the interiors were stained with Alizarin Red S, which stains for calcium carbonate. After staining, the outside of the test and the interior supports were bright red or purple, and were able to be distinguished from other carbonate inside the test that stained to a different, lighter color, and from noncarbonate material inside the test that did not stain. The fully prepared specimens are shown in Figs. 16-20.

a. 

b. 

Figure 16: Photos of internal inspection of specimen GA 14; a. Lateral profile view, b. Top view.



a.

b.

Figure 17: Photos of internal inspection of specimen MS 22; a. Lateral profile view, b. Top view.

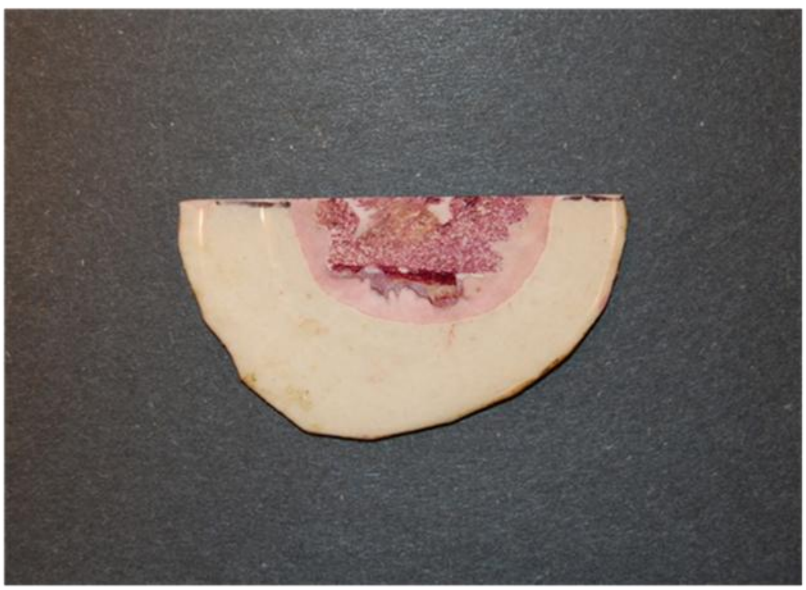
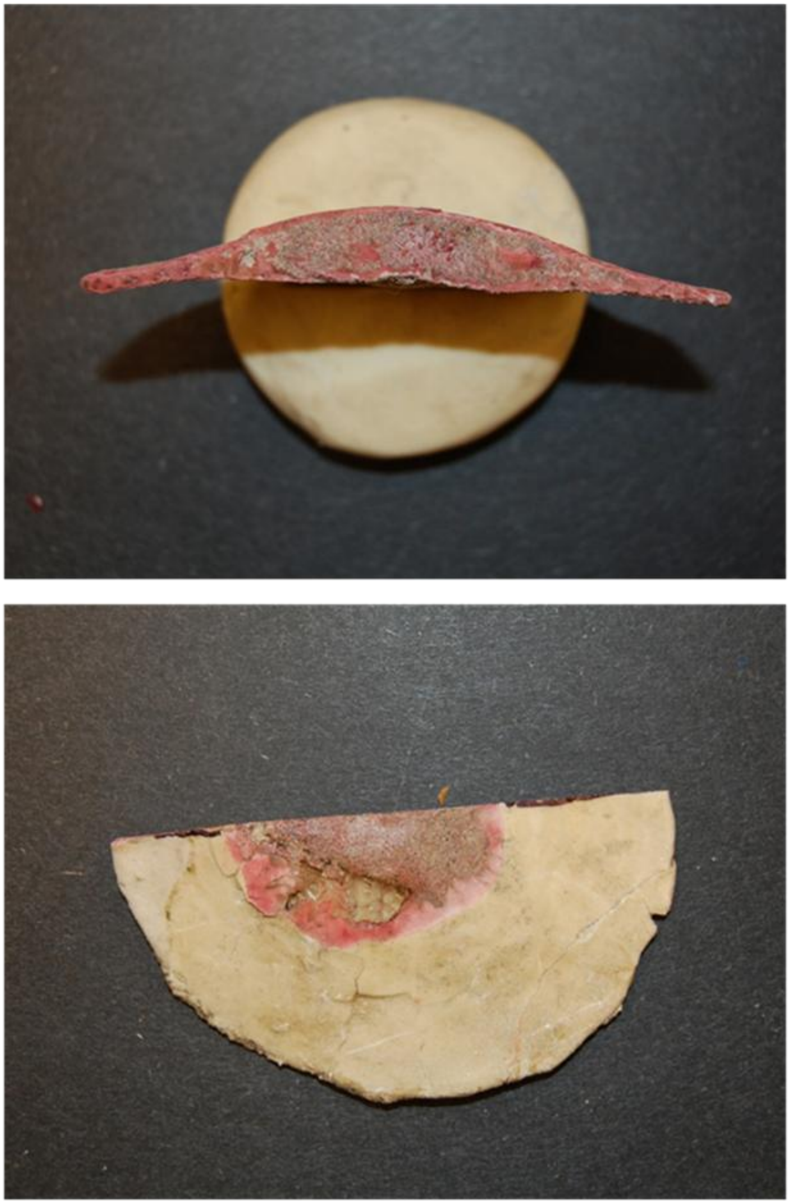


Figure 18: Photos of internal inspection of specimen SC 8; a. Lateral profile view, b. Top view.



a.

b.

Figure 19: Photos of internal inspection of specimen SC 13; a. Lateral profile view, b. Top view.

Figure 20
Photos of internal inspection of specimen NC 18

4.0 RESULTS

4.1 Observations

The profile shapes of *P. lyelli* specimens from North America differ across the geographic distribution of the species. Among specimens collected, *P. lyelli* fossils can be categorized into three different morphotypes (Fig. 21). In this study, morphotype 1 refers to those specimens whose profile is very flat, with little vertical relief. Morphotype 2 refers to those whose profiles are domed, with higher apices than those of morphotype 1. Morphotype 3 refers to those specimens whose profiles have a pointed, or peaked shape. Many, but not all localities contain more than one morphotype.

The apex at the convergence of the ambulacra is the highest point in profile on all specimens. However, the specimen profiles differ below the apex, along the apical disc. In those specimens that represent morphotypes 1 and 2, the profile line leading to the outer edge of the echinoid becomes increasingly convex along the ambulacra. At the end of the ambulacra, the profile flattens out to the outer edge. Depending on the degree of convexity, this creates a flat or slightly domed profile shape.

Conversely, in specimens that represent morphotype 3, the profile line leading to the outer edge of the echinoid becomes increasingly concave, flattening out to the outer edge. This creates a peaked, or pointed profile that differs greatly from many other specimens. Furthermore, the specimens that represent morphotypes 1 and 2 all have different degrees of domed profiles. This could suggest another significant profile shape difference in this category of sand dollar. However, since the differences are not as

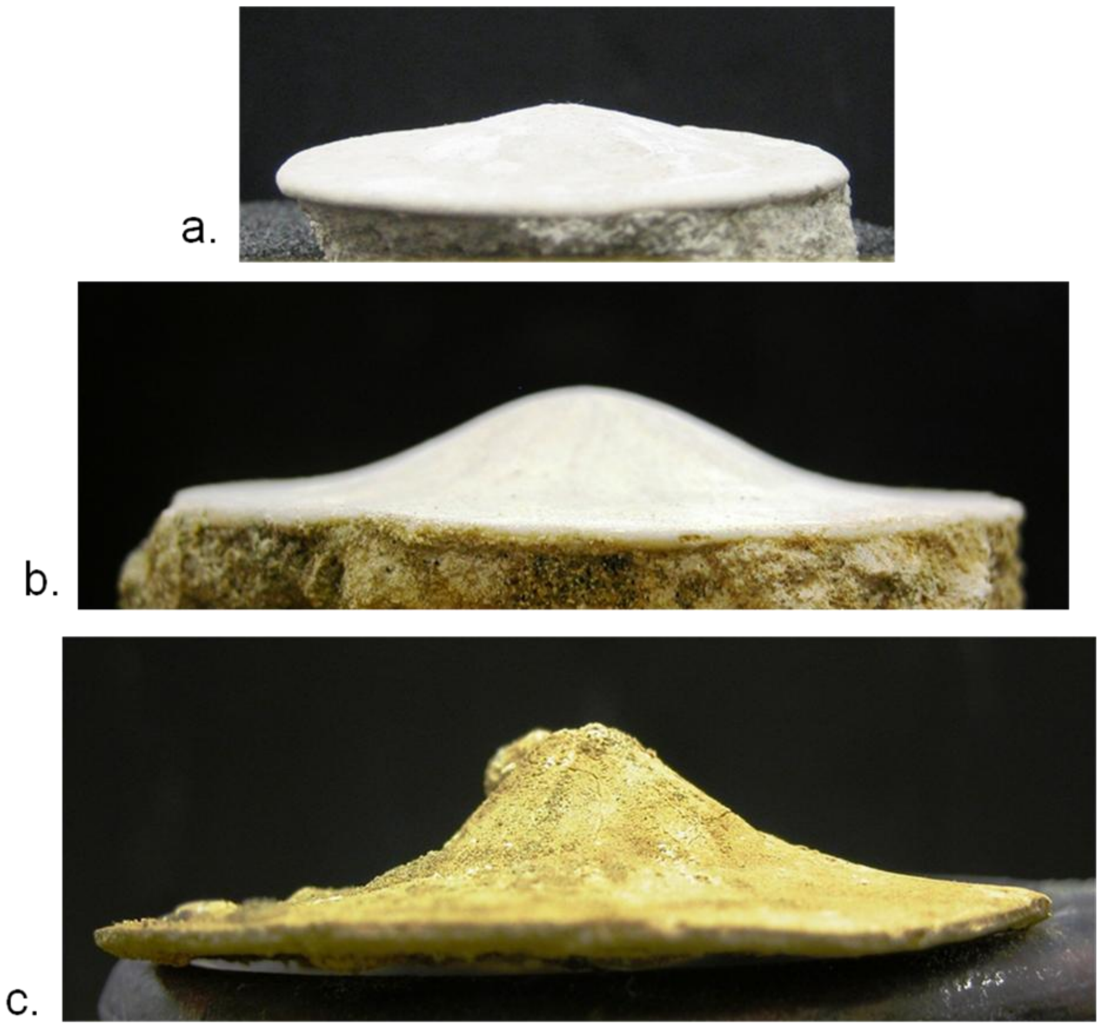


Figure 21
Specimen photograph examples from each morphotype; a. NC 8, morphotype 1; b. SC 15, morphotype 2; c. MS 34, morphotype 3

distinct in observation alone, and most observation has at least some preferential bias and is not very repeatable, quantification and morphometric analysis is necessary to determine if there is truly a difference or not.

4.2 Physical Comparison

When comparing the different morphotypes, certain characteristics of the tests stand out. For example, it seems upon first inspection that morphotype 3 has a much thinner test than the other two profile shape categories. In order to investigate these relationships further, physical measurements were plotted against each other, and categorized to see if there is any pattern associated with morphotype.

In the plot of apical height vs. length (Fig. 22), a pattern is seen in the morphotypes. Morphotypes 2 and 3 plot linearly, showing that as the test diameter gets larger, the apical height increases as well. Morphotype 1, however, plots differently, apart from the linear correlation of morphotypes 2 and 3.

In the plots of apical height vs. thickness of test (Fig. 23), no correlation could be seen. In the plot of ambulacra length vs. diameter (Fig. 24), again, morphotypes 2 and 3 plot on top of one another, while morphotype 1 plots differently. Also, this plot suggests two trends within morphotype 2.

4.3 Multivariate Studies

The graphs created from the statistical analyses show varied results. The results from Elliptical Fourier Analysis on all usable specimens are shown in Figs. 25 and 26. For EFA, PCA 1 removed 20% of the variation among the sample set, while PCA 2 removed 15% and PCA 3 removed 11.8%. These three axes removed significantly more

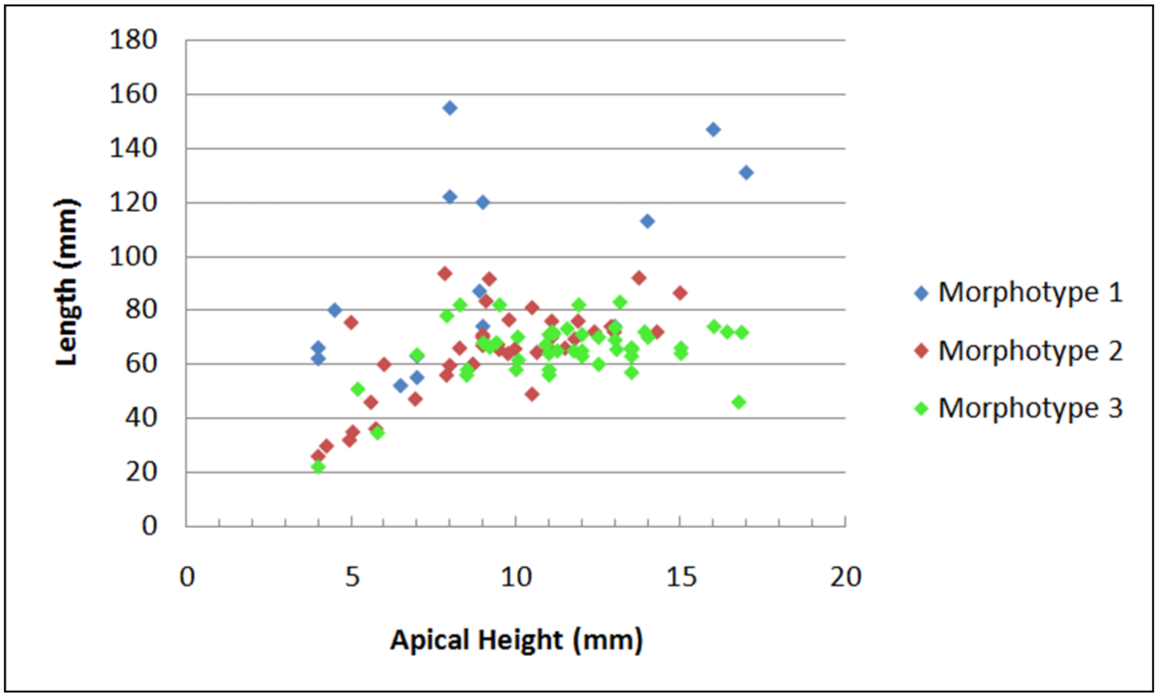


Figure 22: Plot of measurements of Apical Height vs. Length of specimens, categorized by morphotype.

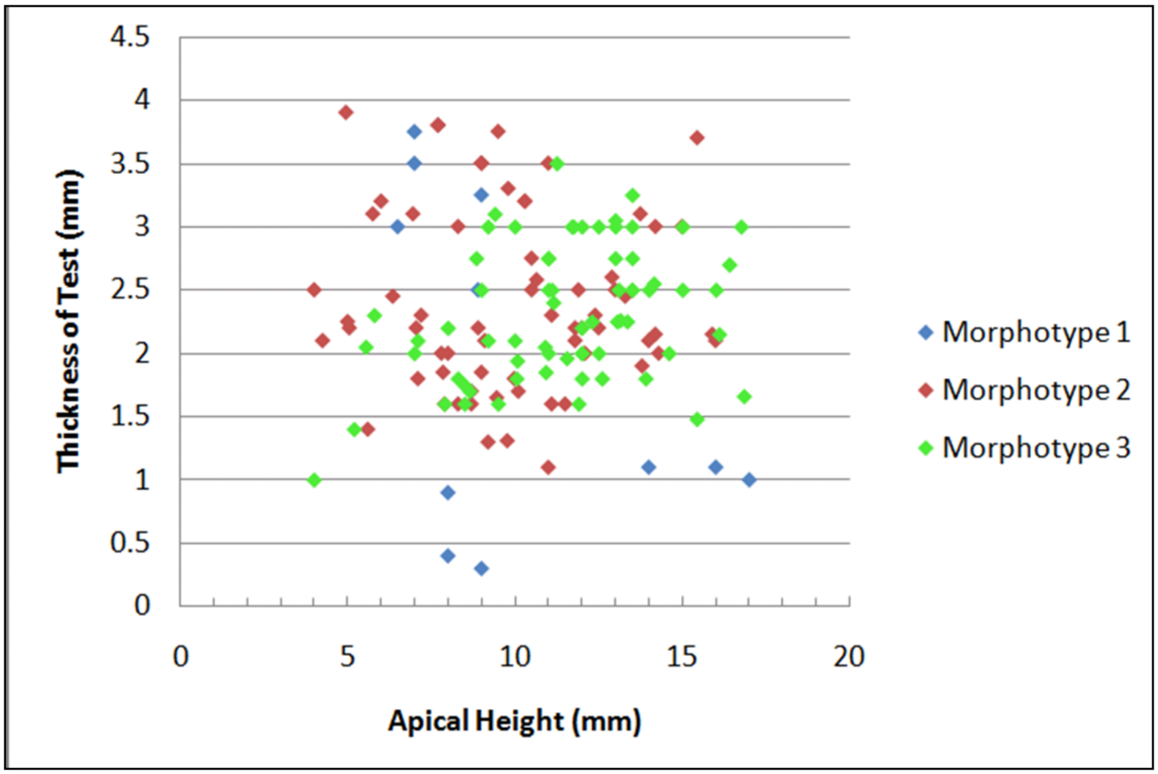


Figure 23: Plot of measurements of Apical Height vs. Thickness of Test, categorized by morphotype.

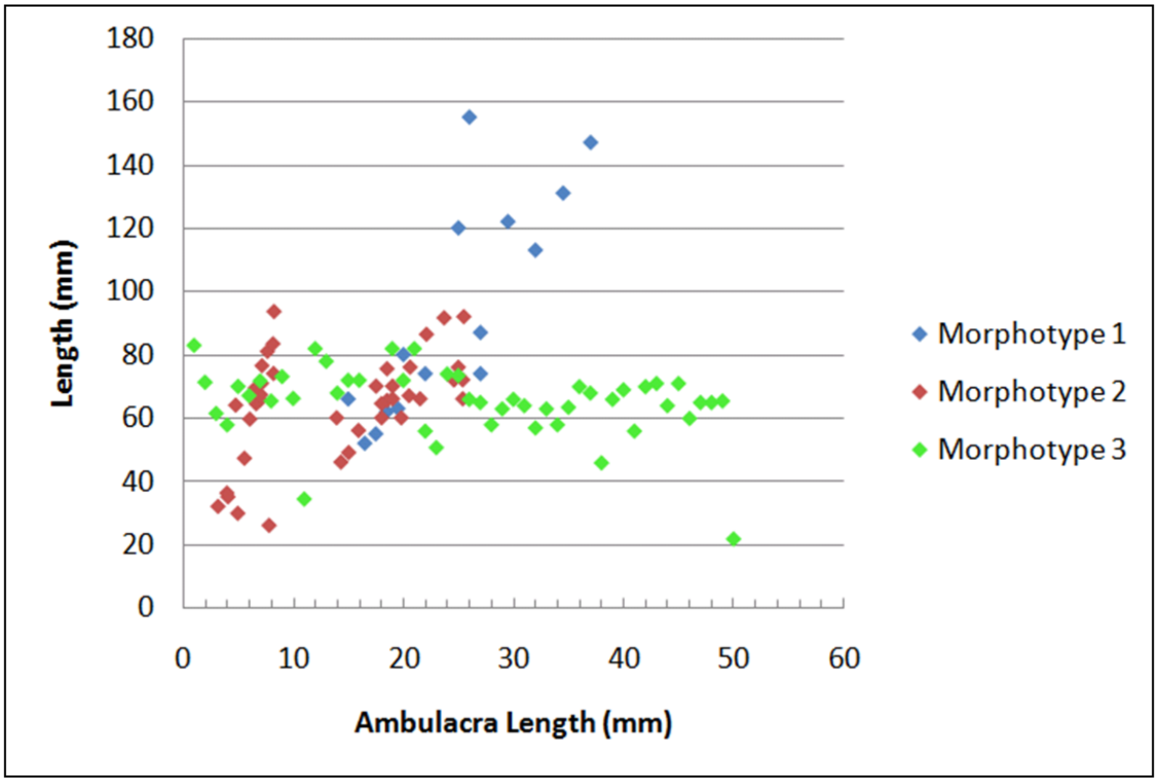


Figure 24: Plot of measurements of Ambulacra Length vs. Length of specimens, categorized by morphotype.

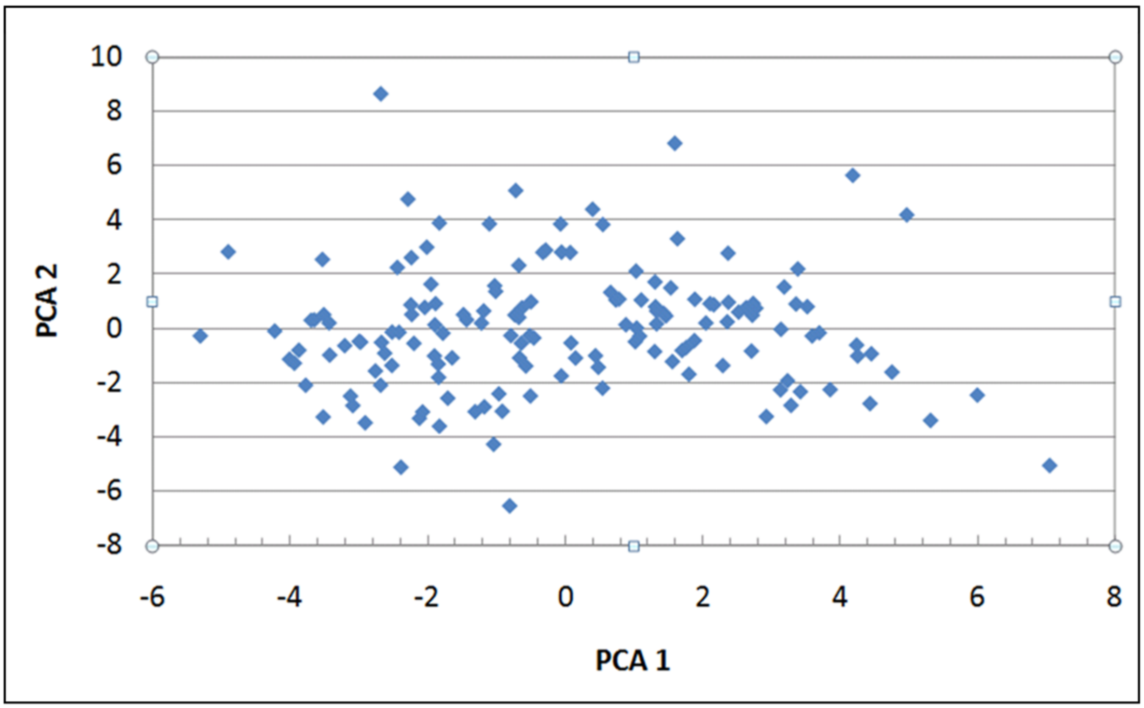


Figure 25: EFA PCA results, PCA 1 vs. PCA 2.

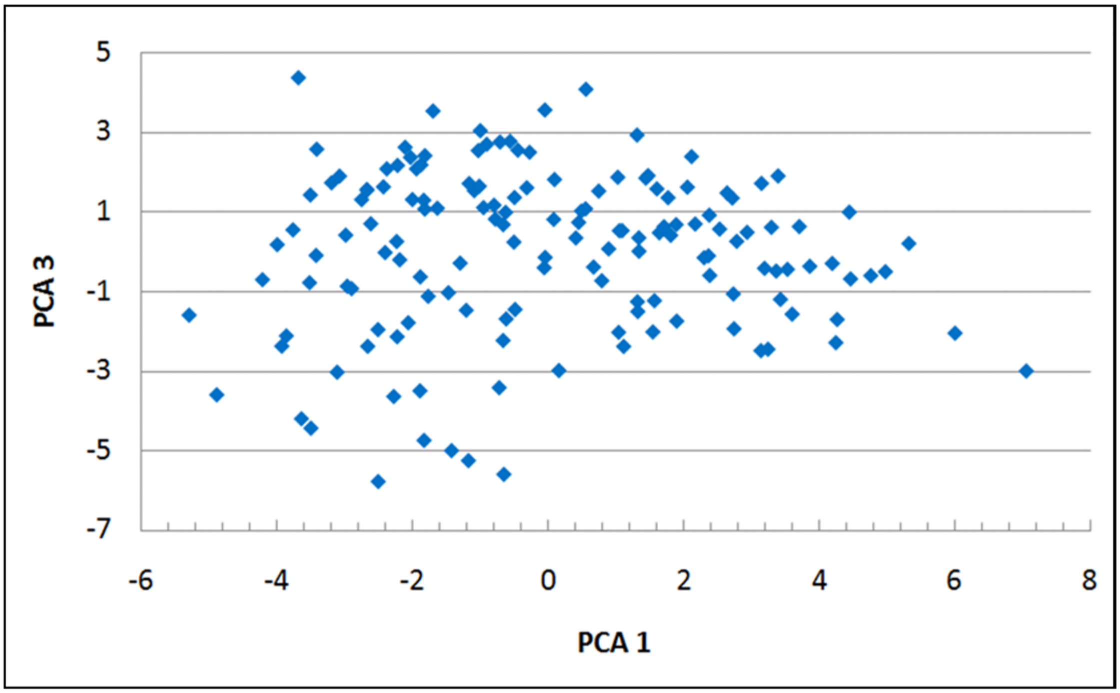


Figure 26: EFA PCA results, PCA 1 vs. PCA 3.

variation than any of the others, and were therefore compared in scatterplots to see if the specimen data produced any distinct clusters. Since PCA had the highest percentage of variation removed, PCA 2 and 3 were plotted against it i
n separate scatterplots.

The results from the Procrustes landmark analysis on all usable specimens are shown in Figs. 27 and 28. For Procrustes, PCA 1 removed 32.8% of the variation among the sample set, while PCA 2 removed 17% and PCA 3 removed 15.4%. As in the EFA results, Procrustes PCA 1, 2 and 3 clearly removed more variation than any of the other axes, and therefore were compared in scatterplots in the same way as were the EFA Principal Component Axes.

The scatterplots for PCA 1, PCA 2, and PCA 3 for each statistical analysis were similarly analyzed in a number of ways. First, the plots were categorized according to state (Figs. 29 - 32). While this was a logical first step, it did not yield unambiguous results. Most specimens plotted over the entire graph, and few distinct clusters could be seen.

However, some patterns could be seen in this categorization. For instance, most specimens from Mississippi and Georgia plotted on top of one another in all graphs for each analysis. Also, on all the plots, those specimens from Florida are clearly separated from those from Mississippi, and, in most cases, Georgia. Other ways of classifying the samples on the plots were developed in order to search for less ambiguous results.

The plots were categorized according to perceived morphotype, as defined by the technique described earlier. Through observation, three distinct profile shapes could be

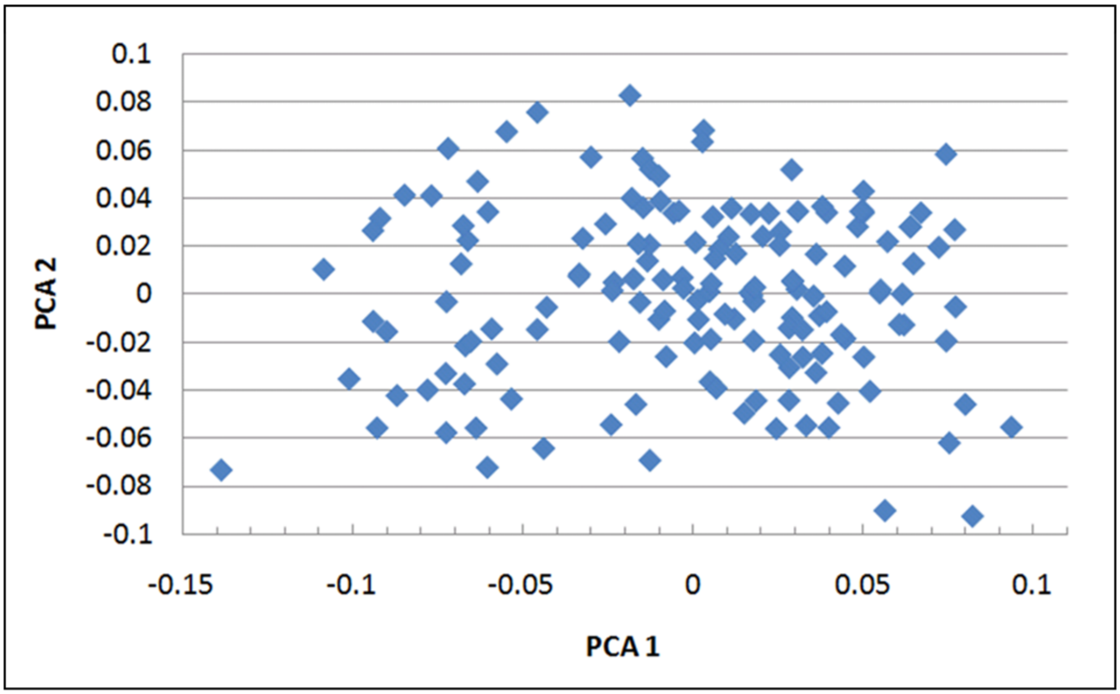


Figure 27: Procrustes PCA results, PCA 1 vs. PCA 2.

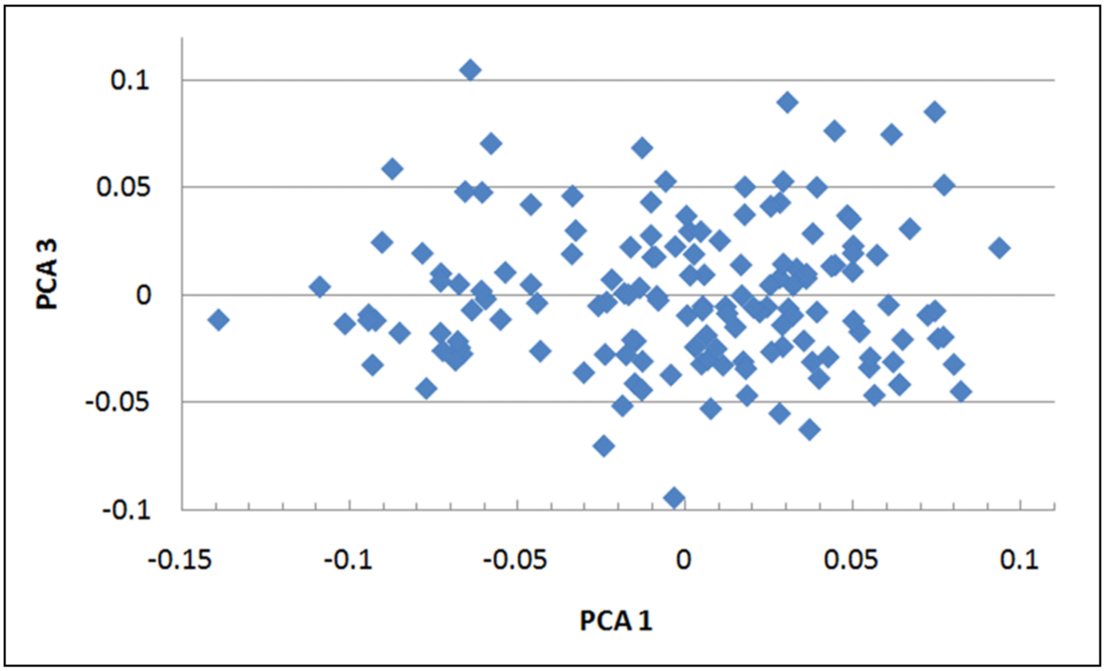


Figure 28: Procrustes PCA results, PCA 1 vs. PCA 3.

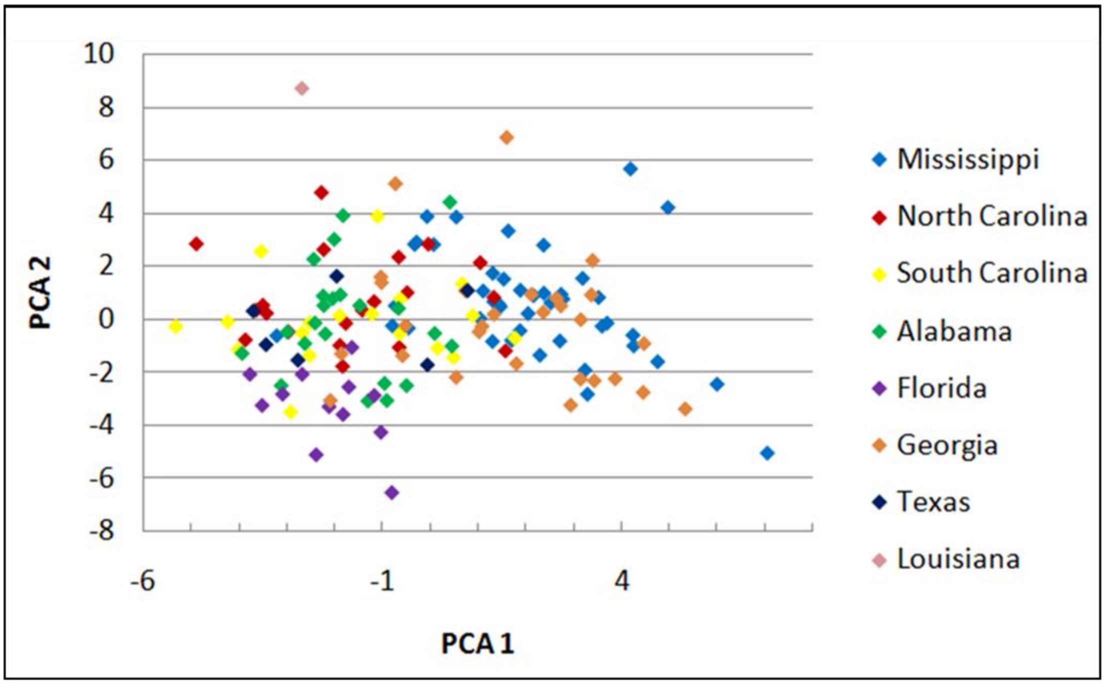


Figure 29: PCA results of EFA (PCA 1 vs. PCA 2), categorized by state.

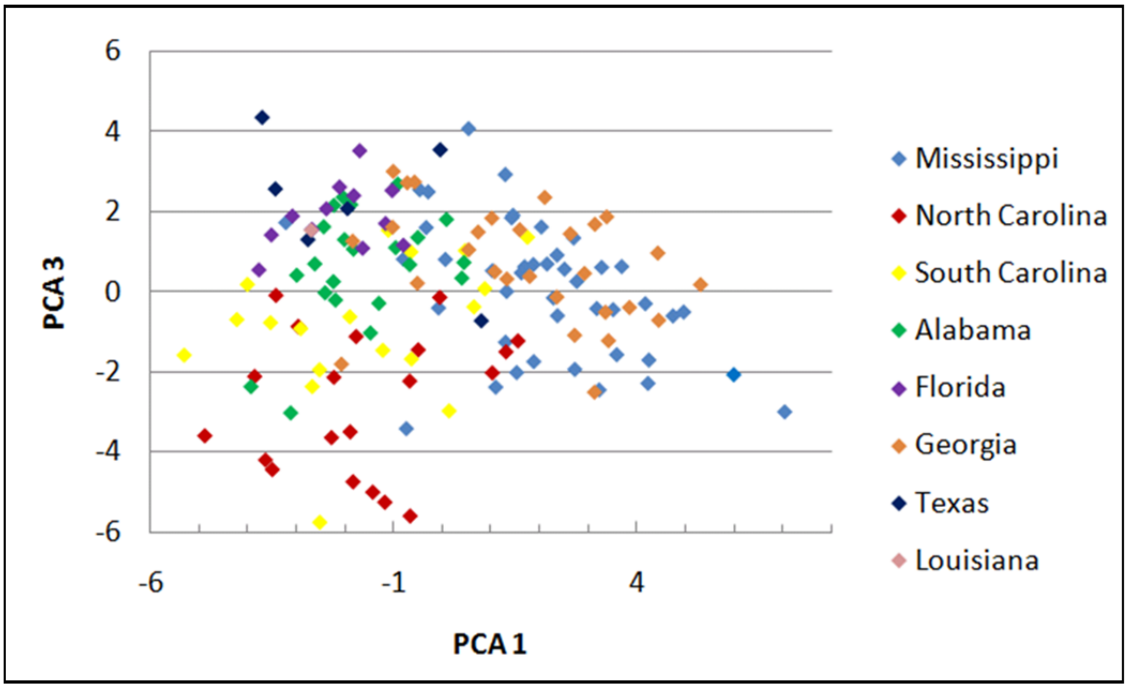


Figure 30: PCA results of EFA (PCA 1 vs. PCA 3), categorized by state.

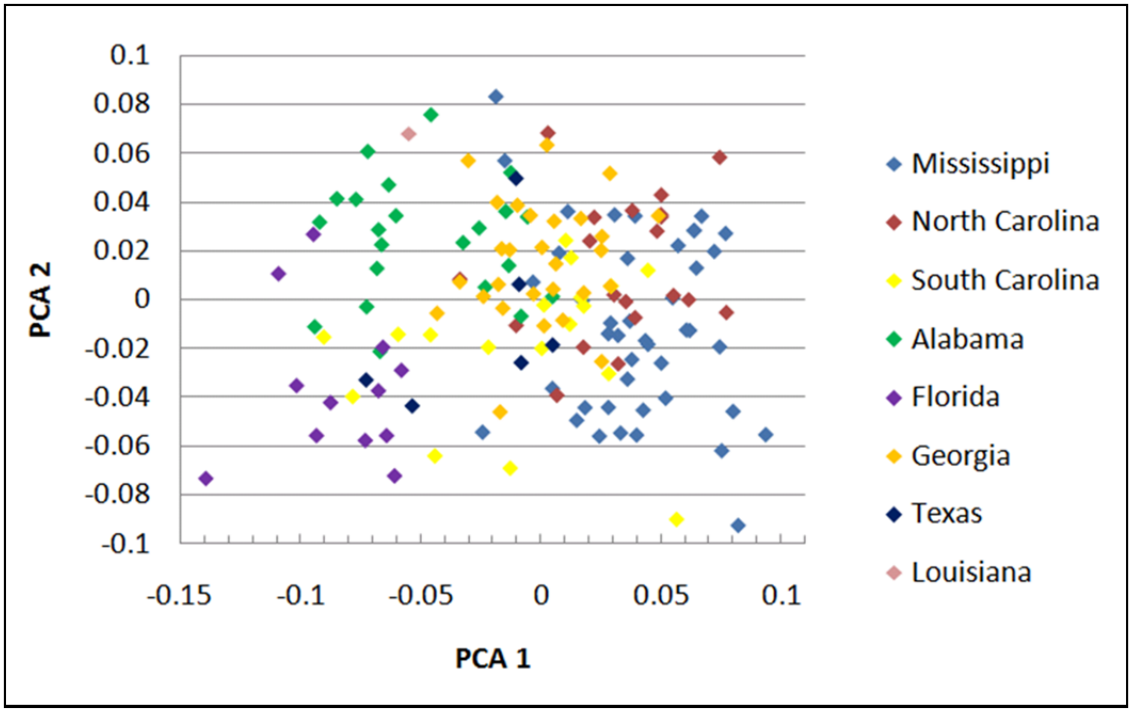


Figure 31: PCA results of Procrustes (PCA 1 vs. PCA 2), categorized by state.

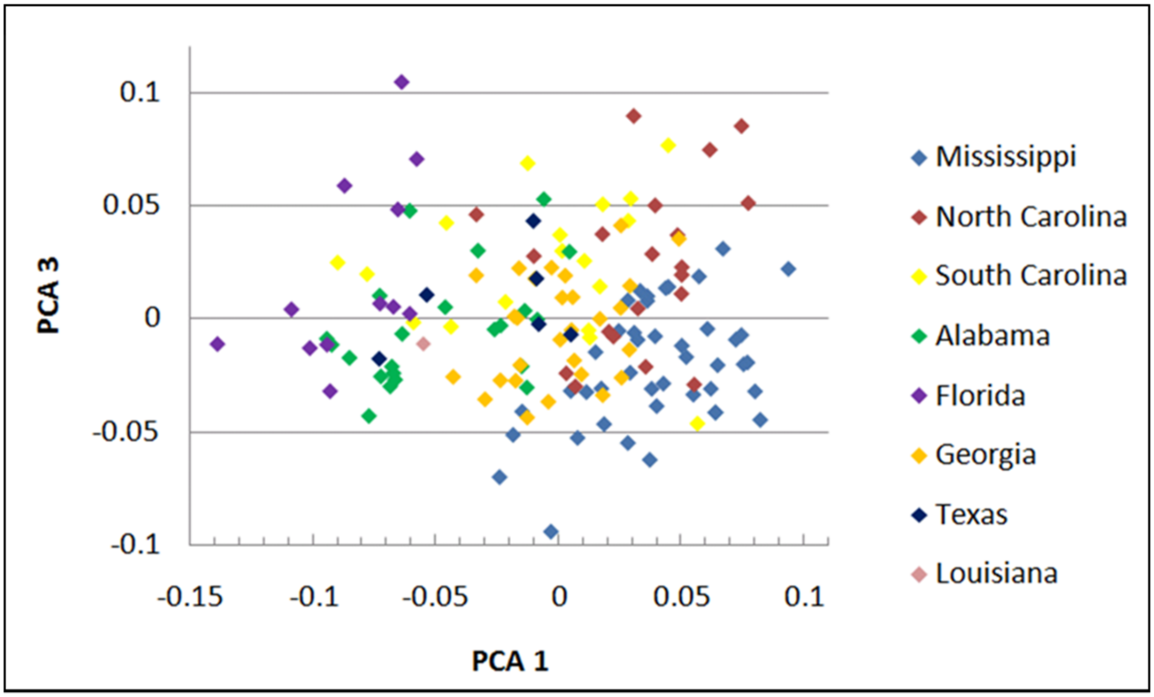


Figure 32: PCA results of Procrustes (PCA 1 vs. PCA 3), categorized by state.

seen. Each specimen was assigned to a morphotype and this information was combined with the PCA data on the scatterplots. The result was clearer than the geographic classification, and showed distinct clusters corresponding to each morphotype (Figs. 33 - 36).

When the plots categorized according to state and those categorized according to morphotype were compared with one another, correlations could not be seen. To show this, the scale of each graph was normalized, and the outline of each morphotype plot was determined. Then, the morphotype outlines were overlain on each state-categorized plot (Figs. 37 – 40). These results show that morphotypes are not set in a specific geographic area.

4.4 Sediment Analysis

The substrate sample from Rattlesnake Bluff, Alabama is a tan, fine to medium-grained, fossiliferous, sandy limestone. According to the location of collection, this sample is from the Gosport Formation. It is poorly-sorted and the grains are moderately-rounded to angular. Close observation of the hand sample reveals many fossils, including echinoids, gastropods, and pelecypods, but few are intact.

In thin section (Fig. 41), after staining using AR5/PF, ~30% of the sample is quartz sand, and is clear in color. ~35% is darker in color, and is most likely altered material or detrital clay. 10% is blue in color which indicates iron-rich calcite or dolomite. Fossils are also pink in color because of their calcitic shells, and comprise ~20% of the sample, with the remaining 5% being void space. The large piece of echinoderm in the thin section shows the cleavage in the calcite crystal clearly.

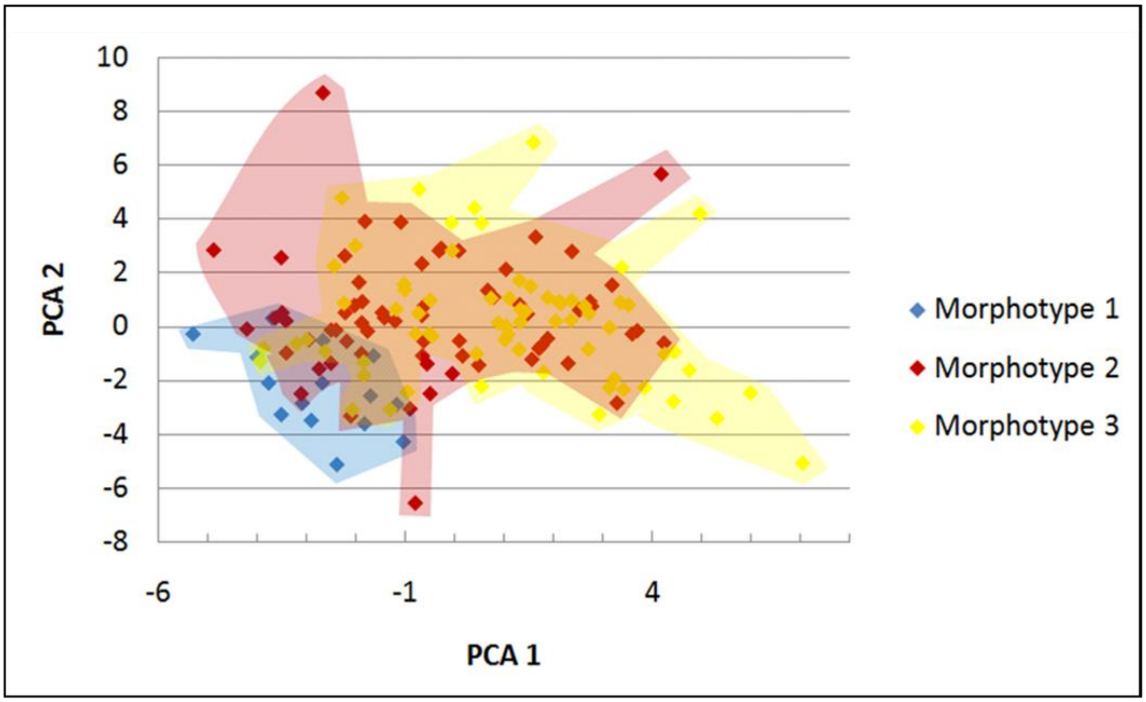


Figure 33: PCA results of EFA (PCA 1 vs. PCA 2), categorized by morphotype.

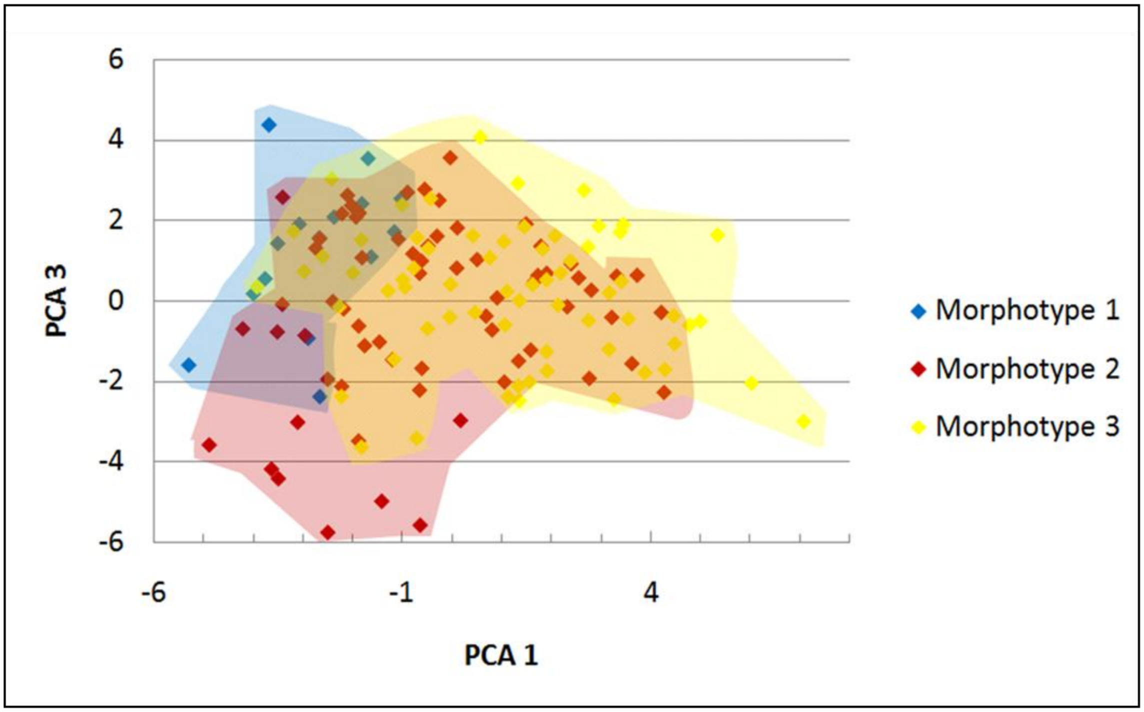


Figure 34: PCA results of EFA (PCA 1 vs. PCA 3), categorized by morphotype.

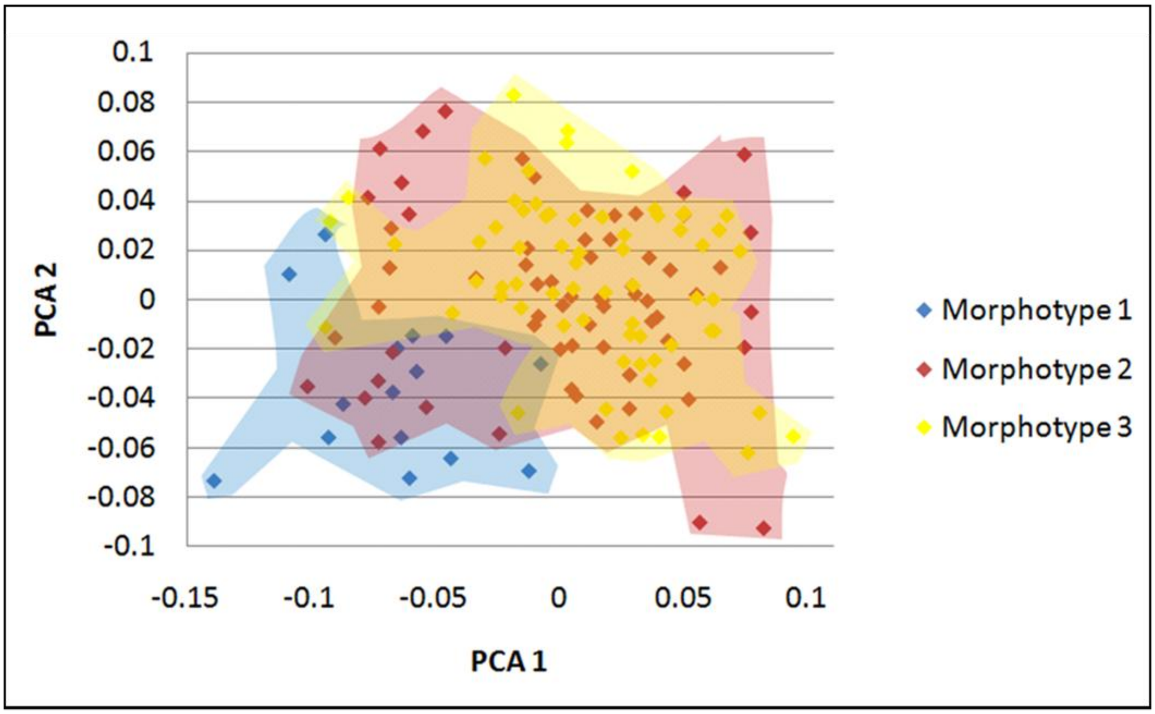


Figure 35: PCA results of Procrustes (PCA 1 vs. PCA 2), categorized by morphotype.

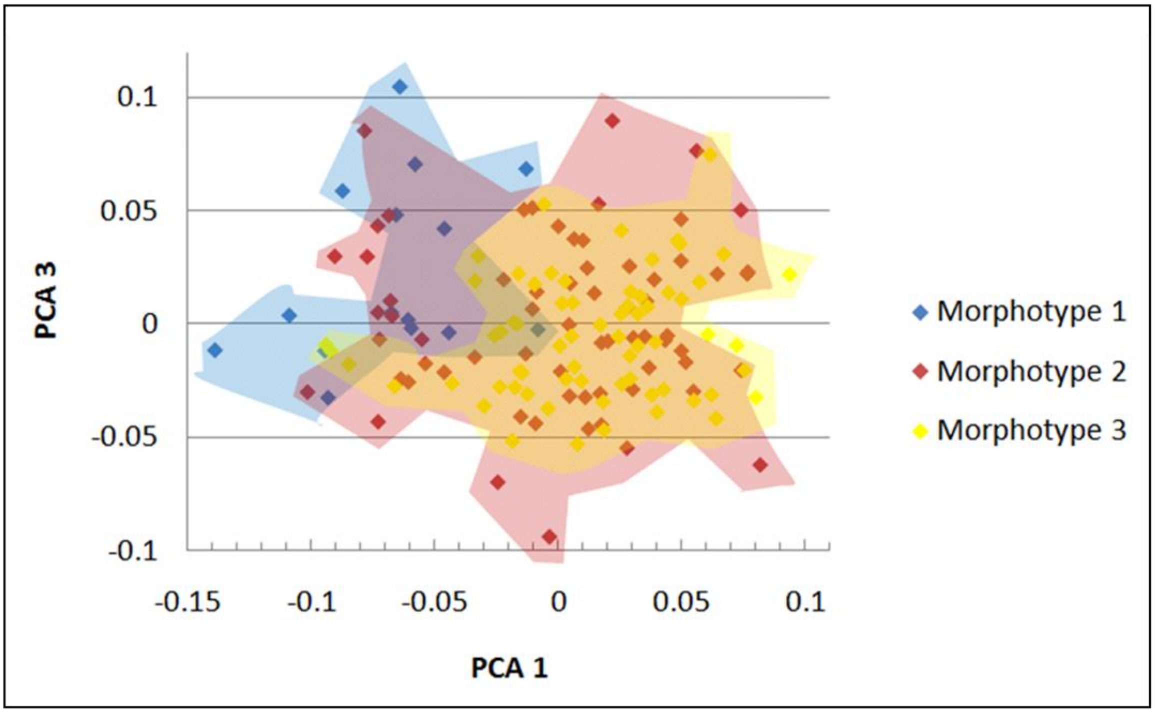


Figure 36: PCA results of Procrustes (PCA 1 vs. PCA 3), categorized by morphotype.

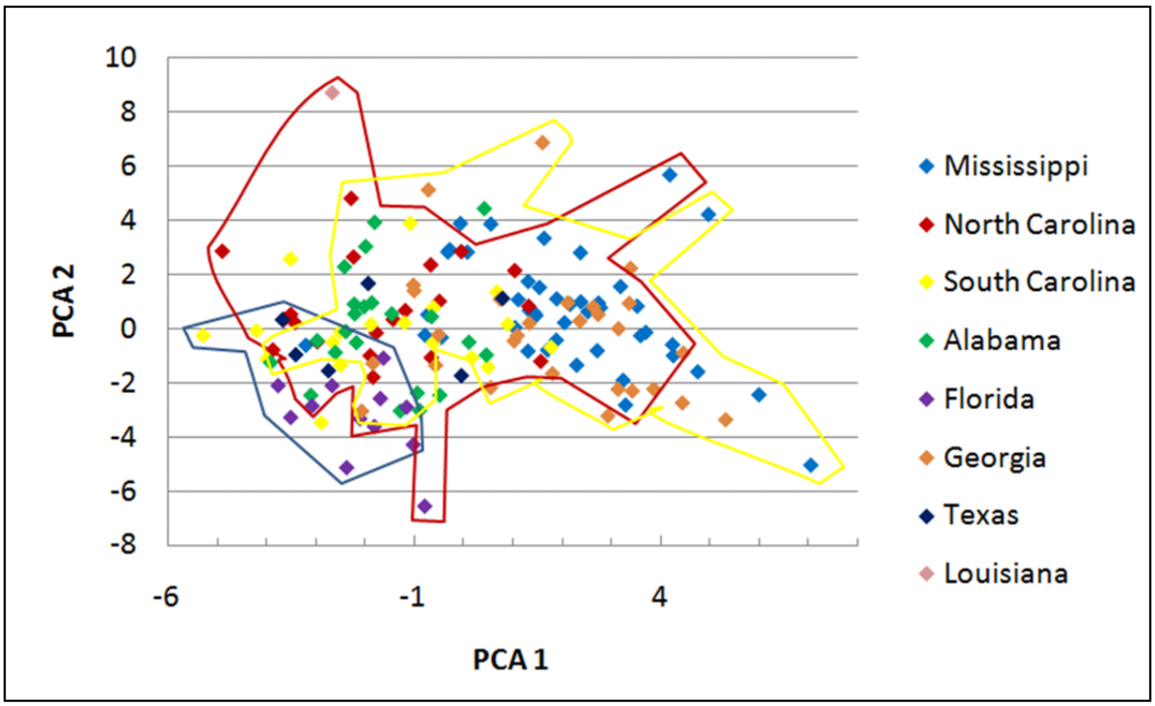


Figure 37: PCA results of EFA (PCA 1 vs. PCA 2), categorized by state and overlain with morphotype data. Blue line encloses morphotype 1, red line encloses morphotype 2, yellow line encloses morphotype 3.

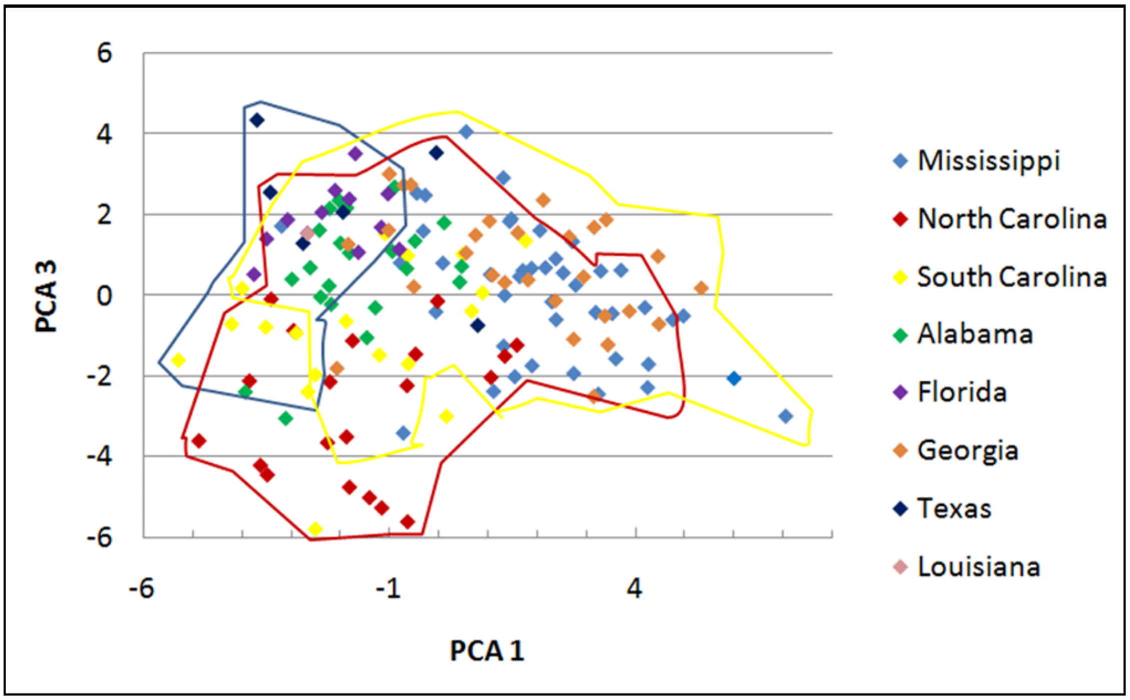


Figure 38: PCA results of EFA (PCA 1 vs. PCA 3), categorized by state and overlain with morphotype data. Blue line encloses morphotype 1, red line encloses morphotype 2, yellow line encloses morphotype 3.

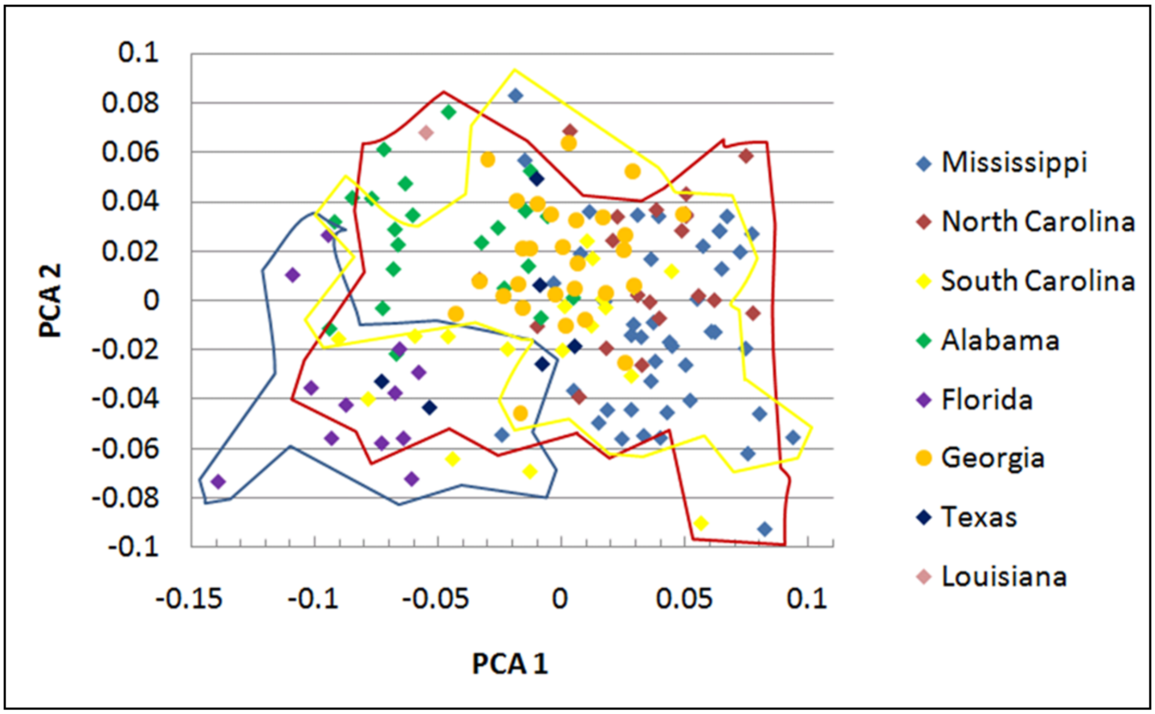


Figure 39: PCA results of Procrustes (PCA 1 vs. PCA 2), categorized by state and overlain with morphotype data. Blue line encloses morphotype 1, red line encloses morphotype 2, yellow line encloses morphotype 3.

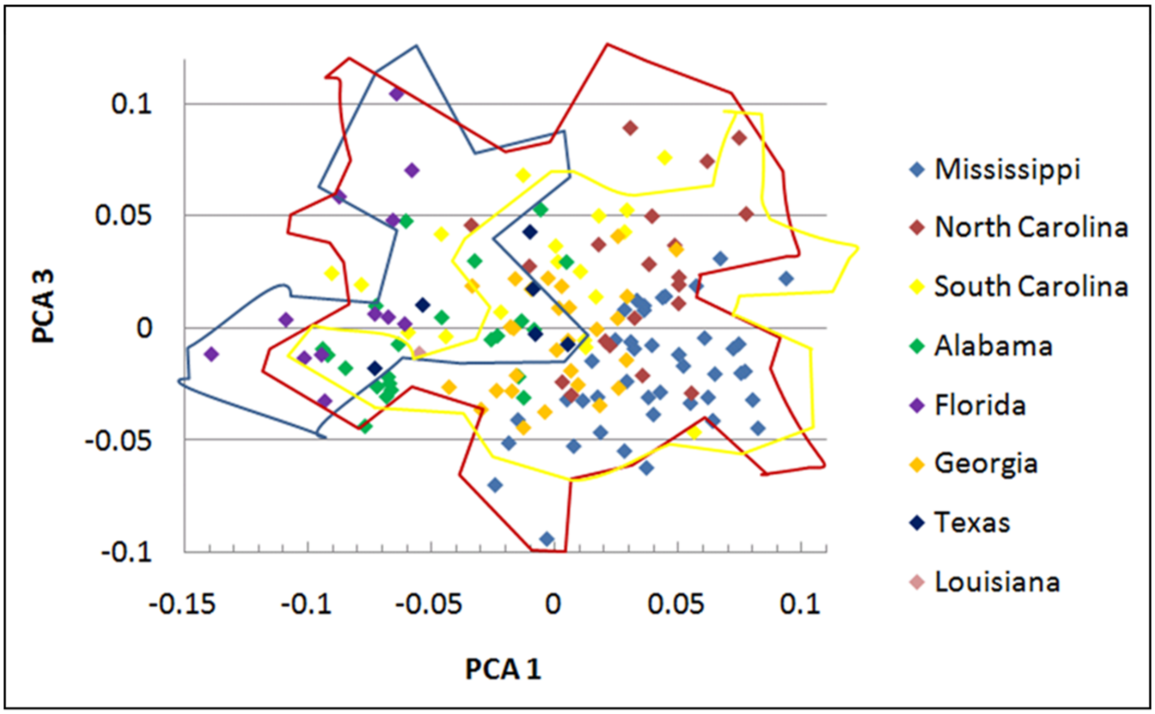


Figure 40: PCA results of Procrustes (PCA 1 vs. PCA 3), categorized by state and overlain with morphotype data. Blue line encloses morphotype 1, red line encloses morphotype 2, yellow line encloses morphotype 3.

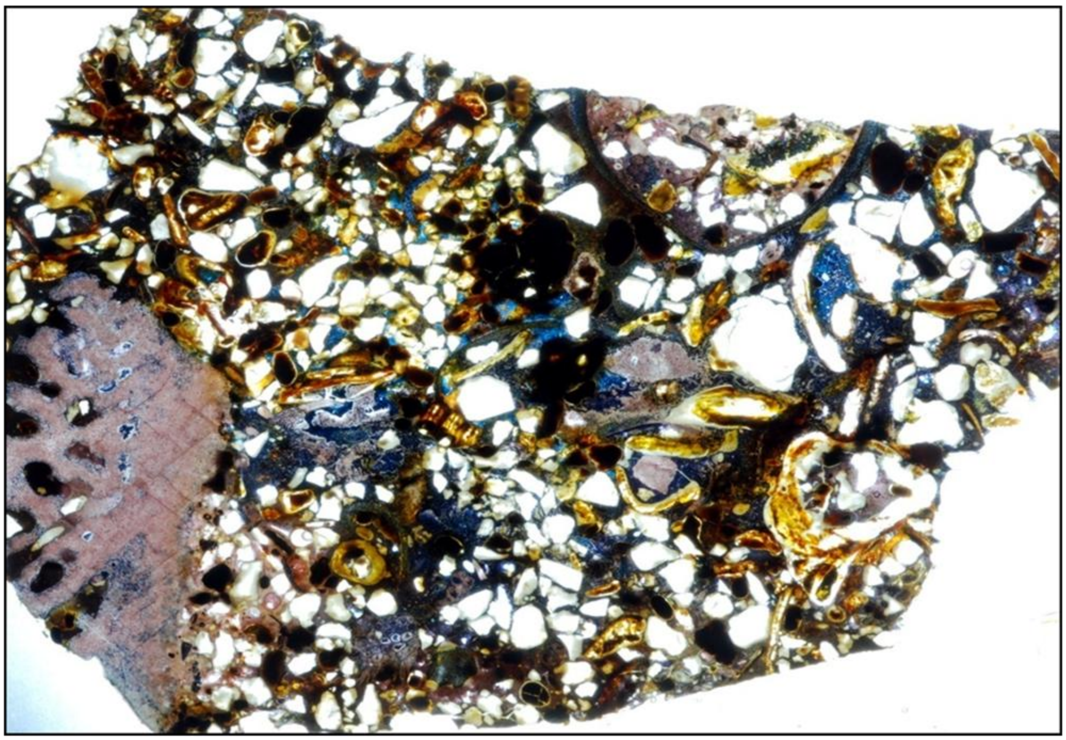


Figure 41: Photo of thin section slide of sediment from collection locality at Rattlesnake Bluff, AL.

Some pelecypod shells are clearly seen as well. Microfossils include many different types of forams. Forms include globular, biseral, triseral, and some trochospiral shapes. This indicates the paleoenvironment to be shallow marine, close to the shore (Armstrong and Brasier, 2005).

The sample at Perry, GA is a white to cream-colored fine to medium-grained fossiliferous limestone. According to the location of collection, this sample is most likely a part of the Tivola Limestone that is found in Georgia and is equivalent to part of the Ocala Limestone found in Florida. It is poorly-sorted and the grains are moderately-rounded to angular. Observation of the hand sample reveals the presence of broken echinoids, bryozoans, pelecypods, and gastropods.

In thin-section (Fig. 42), after staining with AF5/PF, ~80% of the sample is carbonate, mostly calcium carbonate (stained pink), 10% is fossil material which is also colored pink due to its calcareous composition, and 5% is void space. There is also a bit of orange material which is most likely clay. Fossils include echinoderm fragments, gastropods, and bryozoans. Microfossils include many different kinds of forams, which include biseral, triseral, and trochospiral forms. This indicates the paleoenvironmental setting to be shallow marine, close to shore (Armstrong and Brasier, 2005).

4.5 Structural Analysis

Based on the internal inspection of the sectioned specimens, several conclusions can be made. The specimen SC 8 from the Santee Formation in South Carolina shows several substantial supports toward the outer margin. Other than this, not much of a

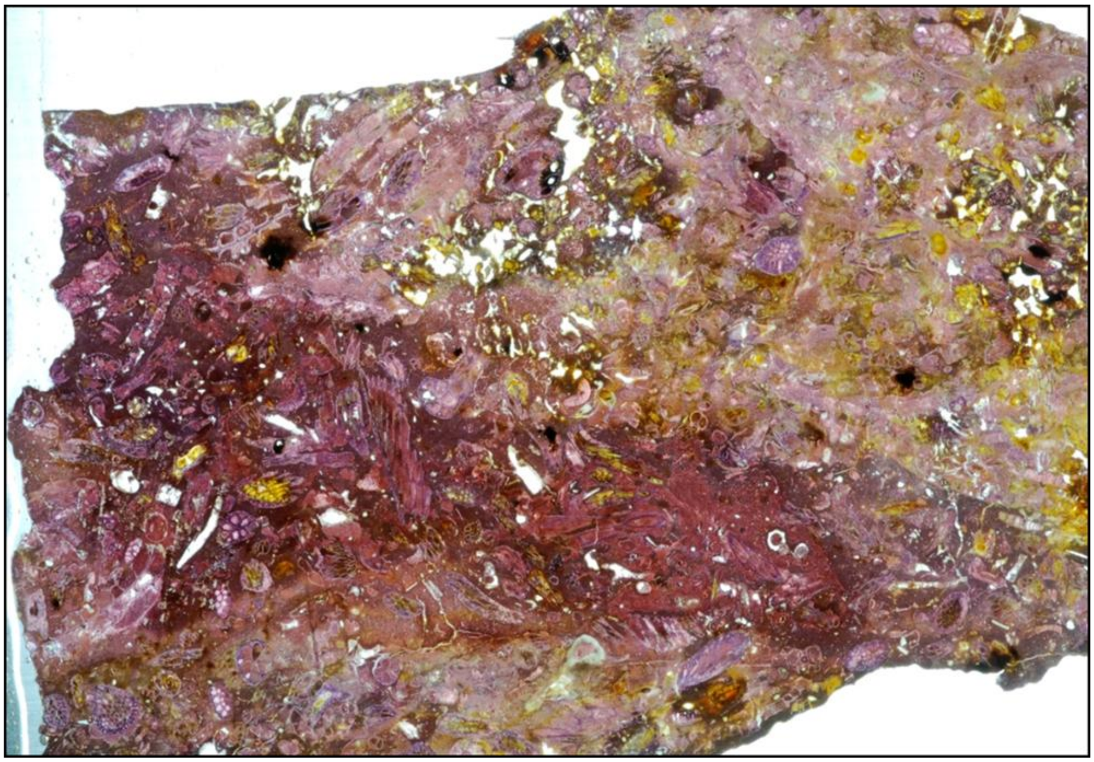


Figure 42: Photo of thin section slide of sediment from collection locality at Perry, GA

pattern of support can be seen. Of the support structures that can be seen in the profile, the largest are beneath each ambulacrum, as is common in sand dollars. This is even more apparent when looking at the slice of the aboral surface.

In the specimen NC 20 from the Castle Hayne Formation in North Carolina, several supports can be seen toward the outer margin. As in specimen SC 8, ambulacral supports can be seen, but no other pattern can be discerned. In the specimen MS 22 from the Moodys Branch Formation, not much can be seen at all. The inside of the test looks to be completely filled with sediment, and any internal structure that was supporting the aboral plate has been displaced, or is obscured. This could be due to the poor preservation, or even possible deformation of this specimen.

The specimen GA 14 from the Tivola Formation presents a puzzling situation. The sediment on the interior of the test looks more quartz-rich than that at the quarry where this specimen was collected. This is clearly shown with the stained profile, in that the Tivola Formation at this locality is almost pure calcium carbonate, and none of the sediment on the inside of the test stained with ARS/PF. The stained support structures of the test stood out against the unstained sediment filling the rest of the interior; and seemed to "float" in the sediment instead of being connected to the oral and aboral plates. Since no connections could be seen, it is difficult to tell where the support structures are in relation to the ambulacra. Also, supports toward the outer margin are unclear.

In the specimen SC 13 from the Santee Formation, the sediment looks to be more quartz-rich than the sediment on the interior of SC 8. The sediment did not stain completely with ARS/PF, and therefore is not completely calcium carbonate. Support structures in the profile slice are not connected to the oral or aboral plates, and again seem to "float" in the sediment. The supports toward the margin were unclear, and no supports could be seen from the aboral slice.

4.6 GIS

Using ArcGIS® and its many components, a dynamic map of the distribution of the highly variable echinoid *P. lyelli* was produced. The multiple data analysis tools available are extremely useful and aid in visualizing the data collected for this paleontological study. Without a map, it is very difficult to interpret the possible geographic and geologic connections and correlations between samples. For instance, the changes in morphology could be geographically based, or tied to the substrate of the area in which the sand dollar lived. Furthermore, the morphology could be tied to an evolutionary change, therefore those that are the most different in shape are also the most different temporally. The GIS map aides in testing all three of these hypotheses, and also, in building the map and going through the steps of geoprocessing, one can gain a better understanding of the geographical area and all of its many components.

From the map generated for this project, it is possible to see the sites that represent specifically one lithology, one age, or one echinoid profile shape. When the data are selected by sediment type, the west has more detrital substrates than the east. Overall, sediment types seem to be concentrated in certain locations. More specifically, the substrate is quartz sand in the Cook Mountain Formation in the west. Eastward, the substrate becomes more calcareous in the Moodys Branch Formation, but retains much quartz sand. Farther east, the substrate becomes pure limestone with some dolostone in the Tivola and Ocala Formations, and finally, pure limestone is the predominant sediment type in the easternmost collection localities, including the Santee and Castle Hayne Formations.

According to the substrate in which the specimens were collected, there seems to be no correlation with profile shape. The defined morphotypes are independent of sediment type and geography.

5.0 DISCUSSION

Both Rattlesnake Bluff, Alabama and Perry, Georgia have similar fossil assemblages, but different sedimentary characteristics. Rattlesnake Bluff is a sandy limestone with a substantial amount of detrital material and a significant amount of porosity, while Perry is a coarser limestone with much less porosity and no quartz. This indicates that each unit had a slightly different environment upon deposition.

Fossils in these sediments indicate shallow marine environments. Echinoids, and mollusks such as gastropods and pelecypods, live nearer to the shore in very shallow marine environments, reinforcing the setting indicated by the forams. Rotaliina forams were found in the Perry, Georgia sample, as well as Globigerina in both the Perry sample and the Rattlesnake Bluff, Alabama sample. Rotaliina has a wide variety of forms and is common in shallow water. Examples found in the Perry sediment include *Bolivina* and *Islandiella*. The presence of *Globigerina* in both sediments indicates shallow water as well.

The differences in sediments could be due to differences in energy of the depositional setting. Quartz sand is found at many different marine depths, but is most abundant at the very nearest points to the shore and actually onshore on beaches and barrier islands. Areas surrounding these environments all produce limey sediments and limestone as well. With all of the fossil and sedimentary information at hand in this study, one would most likely place the sandy limestone of Rattlesnake Bluff closer to shore than the pure carbonate of Perry.

From the observations made of the internal structure, the supports of *P. lyelli* follow the pattern of sand dollars in the type profile of morphotype 2. However, in peaked profiles, like that of morphotype 3, it is not apparent where the support is located for the aboral plate and apical cone. In specimen GA 14, the sediment found on the inside of the test differed from the sediment in which it was collected. One explanation for this situation could be that the specimen was not collected in situ, was transported post-mortem, or re-worked post-burial from a different unit.

The results from the structural analysis reinforce those found from multivariate statistical analyses. Morphotype 1 forms a tighter cluster in all the multivariate analyses than the other two morphotypes, and is separated from the other two in all plots. Morphotypes 2 and 3 can be distinguished from one another in all plots, but are less separated from each other than they are from morphotype 1. This suggests a closer relationship between these two profile shapes. This evidence, combined with a knowledge of the stratigraphic ages across the collection area, indicates the possibility of a gradational change in profile shape over time.

One of the main problems with this study is the fact that each perceived morphotype has been described before, and many do agree that they are different enough to be classified as something other than simply *P. lyelli*. However, no literature has been published, since Cooke (1959) and Kier (1980) synonymized *P. lyelli,* clarifying the taxonomy, and nothing has been done to define formally each subspecies as its own species. Subspecies of *P. lyelli* have been described, used frequently and often incorrectly. Many times, the species name *lyelli* is dropped and the subspecies is referred to as its own species.

This brings up the question of what defines a subspecies. Generally, subspecies are said to arise as a result of geographic isolation or are tied to a specific geographic distribution (Prothero, 2003). These subspecies of *P. lyelli* would be correct if there were physical barriers separating these three morphotypes from each other.

Since no such barriers are present, *P. lyelli* and its two subspecies, *P. lyelli pileussinensus* and *P. lyelli floridanus*, should be reclassified as three distinct species according to the three defined morphotypes. Morphotype 1, having a flat profile shape, is defined by the species *P. floridanus*, and morphotype 2, having the domed profile shape, is defined by the species, *P. lyelli*. Morphotype 3, having a very sharply-pointed profile shape, is defined by the species, *P. pileussinensis*.

5.1 Error Analysis

During the course of this study, several goals of the investigation changed. First, the initial method was to perform statistical analyses to look for a separation between specimens in different states. However, this method was altered during the course of the analysis for several reasons.

After completing the compilation of specimens for the study, it became apparent that several morphotypes were found in any single state, instead of only one morphotype in each, as was earlier thought to be true. In the previous study, involving samples from Mississippi, North Carolina and South Carolina only, this was less apparent, because there was a smaller sample set.

Furthermore, state distinctions and boundaries drawn by government do not always follow geologic boundaries, and, geologic boundaries do not follow state lines. Therefore, organisms cannot be expected to follow those state boundaries, either. So, even though the collection specimen labels are categorized according to the state from which they are collected, that is the only way the state plays into this study.

In addition to this, a scatterplot of PCA results categorized by state shows little separation. For this reason, results were then categorized according to morphotype, yielding better results and showing distinct clustering. Morphotypes are determined by observation, and do introduce a small amount of bias. While this study originated with the intention that no bias would be introduced, this was a necessary step. However, the quantitative results of the scatterplot were not affected, and the morphotypes corresponded to the statistics fairly well. For further analysis, the data were then categorized by sediment type and geography, and comparison of the plots showed little correlation between morphotype and either of the other variables.

Another source of error has to do with the statistical analysis programs. In preparing the photos for SHAPE, it was necessary to edit the background out of the photo. Since this was done by hand, it is possible that some errors were made and the true shape was not clearly represented. Other errors could have occurred during the process of choosing landmark points for the Procrustes analysis. Again, since this was done by hand, it is possible that some landmarks were not precisely placed, and that the variation from the average shape was not correctly calculated. However, much care was taken during these processes, and errors, if any, are minimal.

6.0 CONCLUSIONS

From the results of this study of the morphology of *P. lyelli*, several points are clear. First of all, there are three general morphotypes within the species, *P. lyelli*. Through observation of the specimens over the entire sample set, profile shapes allow the division into three categories: flat (morphotype 1), domed (morphotype 2), and peaked (morphotype 3). This conclusion corresponds to previous published work performed on this species, and division of the species at the subspecies level (Ravenel, 1844; Fischer, 1951; Kier, 1980).

Secondly, the morphotypes are not separated as significantly from one another according to morphometric analysis. The results of the PCA comparison of the Elliptical Fourier Analysis, a scatterplot, show two of the three categories of morphotype overlapping, and one clustering away from the others. This is echoed by the results of the Procrustes analysis, with the PCA scatterplot showing the three categories of morphotype overlapping each other and only morphotype 1 separated slightly from the others.

While it seems logical that these morphotypes could each be associated with a specific sediment type, this does not seem to be the case across the sample set. *P. lyelli* specimens were found in a variety of substrates, but all morphotypes were found in more than one sediment type. There was not a distinction of one morphotype per sediment type. This is reinforced by the scatterplots produced by both multivariate studies. Categorization of the PCA data by sediment type does not produce clustering or clear separation. Also, according to the geographic representation of this study done in ArcGIS, morphotypes are not separated according to sediment type. In the structural analysis, results are not consistent, and in some cases showed a different sediment type internally from that in which they were collected. Overall, there can be no conclusions drawn as to correlation between profile shape and sediment type because of the possibility of specimens being reworked from other units.

Furthermore, there does not seem to be a relationship between profile shape and geography. This is shown in scatterplots for both multivariate analyses which do not show clustering or significant separation when categorized according to geography. Also, the geographic representation of the study in GIS does not show morphotypes specifically in one area or another, rather, a scattering over the entire geographic range.

However, age could play a part in the emergence of different morphotypes. Profiles that are peaked, or those defined by morphotype 3, are found primarily in the Moodys Branch and Tivola Formations, both from the late Eocene. Flat profiles, or those that are defined by morphotype 1, are found in the middle and late Eocene, but domed profiles are found primarily in the middle Eocene, with a few exceptions extending into the late Eocene. This suggests that morphotype 1 lasted the entire time period that *P. lyelli* existed, and lived separately from morphotypes 2 and 3. Morphotype 2 seems to grade into morphotype 3 as time progresses, suggesting a temporal morphocline.

This evidence suggests that these morphotypes could be separate species. Each morphotype is physically different from the other two, and further investigation into morphometric analysis methods should define these differences. The fact that the three morphotypes are scattered geographically suggest that they could have interacted with one another, and yet have a different enough structure to suggest differing substrate depths and living situations. A subspecies, if correctly applied, could refer to populations that are geographically or geologically separated, neither of which is the case with *P. lyelli*. There is no barrier between morphotypes, geologic or geographic.

APPENDIX A: Multivariate Statistical Analyses

A.1 Fourier Analysis:

A Fourier transform is a linear operator that decomposes a function into a continuous spectrum of its frequency components. An Elliptical Fourier Transform decomposes continuous shape outlines into a series of component ellipses, or harmonics. When more harmonics are used, the shape produced from the Elliptical Fourier Transform will be very detailed, whereas if few harmonics are used, little detail will remain. Therefore, with an increasing number of frequencies, there is an increasing amount of detail in the resulting shape (See Figures 1A and 2A). Since the specimens in this study are fairly simple curved shapes, nine harmonics capture the shape accurately. Fourier coefficients control the frequency that makes up the curve, when the coefficients are determined, they can be used to reconstruct the shape of the original object.

Fourier Analysis has been used in many applications. It is well suited for communications equipment design and testing. Fourier expressions can be used to describe any type of wave function. Recently, this technique has been used to describe the shapes of curved surfaces. Foote (1989) presented a study of trilobite cranidia using perimeter-based Fourier Analysis. Foote traced the outlines of the shapes by choosing landmarks evenly spaced along the curve, modeling measurements from each landmark point to a centroid point on the cranidium. He then measured the angle between the first landmark and each subsequent point on the perimeter. Each value was normalized by

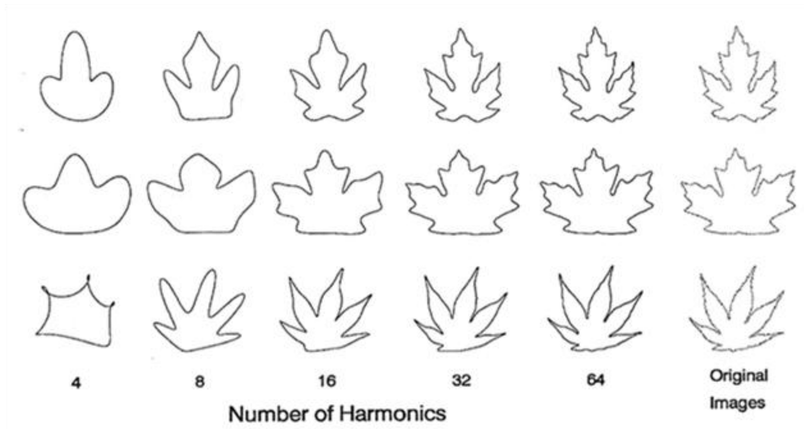


Figure 1A: Example of the amount of detail captured with increasing harmonics in Elliptical Fourier Analysis. From McLellan and Endler, 1998.

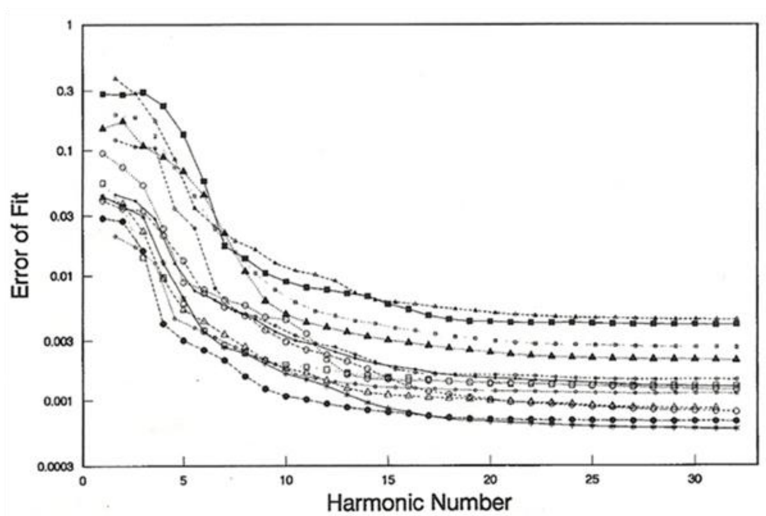


Figure 2A: Graphical representation of the amount of error with increasing number of harmonics used in Elliptical Fourier Analysis. From McLellan and Endler, 1998.

making comparisons to similar measurements on a circle with the same perimeter length as the trilobite cranidium. Normalization is necessary in order to compare homologous points in a set of samples. By normalizing the values, Foote was able to describe the shapes without actually referencing homologous points on each specimen's cranidium.

Foote (1989) described his new method as perimeter-based Fourier analysis. The first part of the procedure is to define a length from the curve's perimeter to a defined point or centroid within the curve. Two variables are then defined. The first is the angle formed as the curve is traced with the defined line, and the second is the radial distance from the defined centroid. The method Foote devised is comparable to Elliptical Fourier Analysis, which is used in this study.

Elliptical Fourier Analysis can be defined as follows:

$$R(L) = \sum_{i=0}^{\infty} a_i\cos(iL) + b_i\sin(iL)$$

$$A(L) = \sum_{i=0}^{\infty} c_i\cos(iL) + d_i\sin(iL)$$

Therefore, the coefficients are defined as:

$$a_i = (2/n)(\sum_{j=1}^{n} R_i\cos(2\pi ij/n))$$

$$b_i = (2/n)(\sum_{j=1}^{n} R_i\sin(2\pi ij/n))$$

$$c_i = (2/n)(\sum_{j=1}^{n} A_i\cos(2\pi ij/n))$$

$$d_i = (2/n)(\sum_{j=1}^{n} A_i\sin(2\pi ij/n))$$

Normalization:

$$R = D/D' \qquad\qquad A = \Phi - \theta$$

D = radius of sample centroid to perimeter

D' = radius of circle

θ = phase angle of specimen (which increases with respect to L)

Φ = phase angle of circle (which increases with respect to L)

L = length along perimeter

In the preliminary study (Williamson, 2006), a computer program was used to perform the Elliptical Fourier Analysis on the sand dollar cross-sectional outlines. The program used in this study is SHAPE (Iwata and Ukai, 2002). This program scans each shape from a bitmap file and translates it into a mathematical expression, or chain code. Once the chain code is created, the program fits a number of ellipses to best approximate the shape. The resulting shape is a combination of ellipses that are expressed as Fourier equations. The coefficients of each term in the Fourier equations represent the size and location of each ellipse. The coefficients therefore form the output data from each cross-sectional outline. The SHAPE program outputs a quantitative map of the outline.

A.2 Principal Component Analysis:

Simply stated, Principal Component Analysis (PCA) is a multivariate statistical technique used to simplify complex data sets, by reducing multi-dimensional data to lower dimensions. Mathematically, PCA is a linear transformation that transforms data to a new coordinate system. Within the new coordinate system the greatest variance of the data lies on the first coordinates or first principal coordinate, the second greatest variance on second coordinate, and so on.

A Correlation Matrix is an m x m symmetrical matrix containing the correlation coefficients between every pair of species. Correlation assesses the tendency of one measure to vary in relation with another. A tendency to change in the same direction is a positive correlation. A tendency to change in opposite directions is a negative correlation. If one measure changes and another does not, it is said to have a zero correlation. The correlation coefficient, r, is the standard measure of correlation and basically measures the shape of an ellipse of plotted points. In two dimensions, an ellipse that is very thin, or close to a straight line would represent a high correlation, while a circle would represent no correlation. In some cases, two-dimensional factor analysis, or literally "factoring" the matrix is all that is necessary. This involves removing common multipliers of all terms and searching for the factors that contain the most information between the two simple dimensions. However, many more complicated cases involve more than two measurements, and more than two or three dimensions. In three dimensions this concept is more difficult to visualize, but the concept is the same. When multidimensional analysis is called for, a matrix of correlation coefficients is used.

The Principal Component Axes are then drawn to include the highest amount of variation possible with each line. PCA1 is drawn along the long axis of a football-shaped ellipse, for instance. Then PCA2 is drawn perpendicular to PCA1, resolving more of the remaining variation than any other line that could be drawn perpendicular to the first.

In this study, the program PC-ORD was used to analyze the Fourier coefficients.

PC-ORD is a computer program that takes a specifically formed matrix of values and displays the PCA results of these values graphically. Using PC-ORD, variances are then plotted on a graph so that they can be visualized. Each data point on the graph represents a specimen, and data points that are positioned closer together are more similar than those that are positioned farther apart.

APPENDIX B: Physical Measurements

Table B1: Physical Measurements

All Measurements in Millimeters			CBD = Cannot be Determined			
Specimen	**Apical Height**	**Thickness of Test**	**Size of Ambulacra**	**Peristome--Edge**	**Periproct--Edge**	**Diameter**
MS1	13.3	2.45	7.65	CBD	CBD	CBD
MS2	10.1	1.7	7.05	CBD	CBD	CBD
MS3	13.35	2.25	7.2	CBD	CBD	CBD
MS4	12	2.2	CBD	CBD	CBD	CBD
MS5	12.5	2.2	6.65	CBD	CBD	CBD
MS6	11.7	3	7.3	CBD	CBD	CBD
MS7	13	3.05	6.9	CBD	CBD	CBD
MS8	16.1	2.15	8.1	CBD	CBD	CBD
MS9	15.9	2.15	7.05	CBD	CBD	CBD
MS10	11.8	2.1	11.55	CBD	CBD	CBD
MS11	8.65	1.7	5.8	CBD	CBD	CBD
MS12	14.2	2.15	7.2	CBD	CBD	CBD
MS13	12.3	2.25	7.05	CBD	CBD	CBD
MS14	10.92	1.85	5.1	CBD	CBD	CBD
MS15	13.1	2.5	7.5	CBD	CBD	CBD
MS16	14.6	2	7.8	CBD	CBD	CBD
MS17	13.8	1.9	6.8	CBD	CBD	CBD
MS18	13.15	2.26	7.82	41.52	25.3	83.04
MS19	8	2	6	29.8	17.55	59.6
MS20	9	1.85	7.05	35.45	CBD	70.9
MS21	11.15	2.4	7	35.7	20.85	71.4
MS22	10.07	1.94	7.3	30.78	18.55	61.56
MS23	8.52	1.74	6.82	28.98	17.25	57.96
MS24	9.98	1.8	6.93	32.86	18.22	65.72
MS25	10.65	2.58	6.57	32.21	19.86	64.42
MS26	10.05	1.8	9	35.05	20.55	70.1
MS27	11.8	2.2	6.5	34.7	CBD	69.4
MS28	10.9	2.05	6.9	33.6	6.5	67.2
MS29	16.84	1.66	6.14	35.91	CBD	71.82
MS30	13.05	2.25	7.15	32.75	CBD	65.5

Table B1 (Continued): Physical Measurements. *Specimen removed from study.

All Measurements in Millimeters			CBD = Cannot be Determined			
Specimen	Apical Height	Thickness of Test	Size of Ambulacra	Peristome–Edge	Periproct–Edge	Diameter
MS31	15.43	1.48	7.23	CBD	CBD	CBD
MS32	12.9	2.6	8.15	37	CBD	74
MS33	9.77	1.31	4.7	32	CBD	64
MS34	12	1.8	7.15	CBD	CBD	CBD
MS35	7.85	1.85	8.2	46.8	35	93.6
MS36	14.15	2.55	8.3	CBD	CBD	CBD
MS37	12.6	1.8	9.3	CBD	CBD	CBD
MS38	9.46	1.65	6.98	33.65	18.53	67.3
MS39	10	2.1	CBD	CBD	CBD	CBD
MS40	11.55	1.96	6.75	36.59	22.21	73.18
MS41	14.2	3	7	CBD	CBD	CBD
MS42	15.45	3.7	8.25	CBD	CBD	CBD
MS43	9.2	2.1	6.25	33.15	CBD	66.3
NC 1	10.5	2.75	15	24.5	14.5	49
*NC 2	6	2	10	18	9	36
NC 3	4	2.5	7.75	13	6.5	26
NC4	16	2.1	7.8	CBD	CBD	CBD
NC5	8	2.2	4.95	CBD	CBD	CBD
NC6	10.3	3.2	4.65	CBD	CBD	CBD
NC7	7.1	1.8	4.7	CBD	CBD	CBD
NC8	6.35	2.45	3.2	CBD	CBD	CBD
NC9	7.7	3.8	4.8	CBD	CBD	CBD
NC10	8.3	3	3.8	CBD	CBD	CBD
NC11	7.7	3.8	4.8	CBD	CBD	CBD
NC12	5.55	2.05	CBD	CBD	CBD	CBD
NC13	7.8	2	3.5	CBD	CBD	CBD
NC14	7.2	2.3	CBD	CBD	CBD	CBD
NC15	7.1	2.1	3.5	CBD	CBD	CBD
NC16	5.8	2.3	3.7	17.3	CBD	34.6
NC17	9.2	3	5.1	CBD	CBD	CBD

Table B1 (Continued): Physical Measurements. *Specimen removed from study.

All Measurements in Millimeters			CBD = Cannot be Determined			
Specimen	Apical Height	Thickness of Test	Size of Ambulacra	Peristome–Edge	Periproct–Edge	Diameter
NC18	8.9	2.2	4.8	CBD	CBD	CBD
NC19	12	2.2	4.8	CBD	CBD	CBD
NC20	8.85	2.75	4.1	CBD	CBD	CBD
NC21	7.05	2.2	4.15	CBD	CBD	CBD
SC 1	9	3.25	22	37	22	74
SC 2	11	3.5	18	32.25	16.75	64.5
SC 3	7	3.5	19.5	31.5	20	63
SC 4	7	3.75	17.5	27.5	16.5	55
SC 5	6.5	3	16.5	26	15.5	52
SC 6	9.5	3.75	18.5	32.75	21	65.5
SC 7	9	3.5	20.5	33.5	22	67
SC 8	9	3.5	19	35	20	70
SC9	10.5	2.5	7.6	40.5	25	81
SC10	5.75	3.1	3.9	18.1	10	36.2
SC11	4.25	2.1	4.9	14.9	8.8	29.8
SC12	14	2.1	7.05	CBD	CBD	CBD
SC13	9.1	2.1	8.1	41.7	17.4	83.4
*SC 14	14.5	2	8.5	CBD	CBD	CBD
SC15	6.95	3.1	5.5	23.6	18.9	47.2
SC16	9.8	3.3	7.1	38.25	22.6	76.5
SC17	4.95	3.9	3.1	16	CBD	32
SC18	12.1	2	6.95	CBD	CBD	CBD
SC19	5.05	2.2	4	17.5	9.95	35
AL 1	11.1	1.6	17.5	35	17	70
AL 2	9.5	1.6	15.9	41	25	82
AL 3	7.9	1.6	15.9	39	20	78
*AL 4	9.4	1.6	15.1	30	19	60
AL 5	9.4	3.1	20.6	34	19.5	68
AL 6	16.4	2.7	26.9	36	21.3	72
AL 7	11.1	2.5	21.4	36	24	72

Table B1 (Continued): Physical Measurements. *Specimen removed from study.

All Measurements in Millimeters			CBD = Cannot be Determined			
Specimen	**Apical Height**	**Thickness of Test**	**Size of Ambulacra**	**Peristome--Edge**	**Periproct--Edge**	**Diameter**
AL 8	11.9	1.6	23.8	41	22.3	82
AL 9	14.3	2	24.6	36	22.3	72
AL 10	11.1	2.3	20.6	38	24.7	76
AL 11	8.7	1.6	13.9	30	10.5	60
AL 12	7.9	1.6	15.9	28	17.8	56
AL 13	13.9	1.8	26.2	36	20.7	72
***AL 14**	14	1.9	23.8	33	22.3	66
AL 15	13	2.5	25.4	36	23	72
AL 16	11.9	2.5	25	38	20	76
AL 17	11	1.1	25.4	33	23.5	66
AL 18	8.3	1.8	17.4	41	30	82
AL 19	8.5	1.6	18.2	28	15.5	56
AL 20	5.2	1.4	11.5	25.4	15	50.8
AL 21	5.6	1.4	14.3	23	11.3	46
***AL 22**	11.7	1.6	22.2	36	22	72
AL 23	12.4	2.3	24.6	36	21.2	72
AL 24	8.3	1.6	19	33	18	66
AL 25	8.7	1.7	19.8	30	16.3	60
FL 1	9	0.3	25	CBD	CBD	120
FL 2	8	0.4	26	CBD	CBD	155
FL 3	17	1	34.5	CBD	CBD	131
FL 4	16	1.1	37	66?	37	147
FL 5	8	0.9	29.5	CBD	CBD	122
FL 6	14	1.1	32	CBD	CBD	113
FL 7	13	CBD	27	CBD	CBD	74
FL 8	4.5	CBD	20	CBD	CBD	80
FL 9	5	CBD	18.5	CBD	CBD	75.5
FL 10	6	CBD	18	CBD	CBD	60
FL 11	4	CBD	15	CBD	CBD	66
FL 12	4	CBD	18.5	CBD	CBD	62

Table B1 (Continued): Physical Measurements. *Specimen removed from study.

All Measurements in Millimeters			CBD = Cannot be Determined			
Specimen	Apical Height	Thickness of Test	Size of Ambulacra	Peristome–Edge	Periproct–Edge	Diameter
GA 1	11.5	2.25	21.5	33	21	66
GA 2	16	2.5	21	37	19.5	74
GA 3	13	2.75	23.5	36.75	23.5	73.5
GA 4	13.5	3	21.5	33	19.5	66
GA 5	11.75	3	22	32.5	19	65
GA 6	11	2.75	21.5	29	18	58
GA 7	13.5	2.5	19.75	31.5	19.5	63
GA 8	13.5	3.25	23	33	20	66
GA 9	15	2.5	21	32	21	64
GA 10	13.5	2.5	21.25	28.5	19	57
GA 11	12	3	21	31.5	20	63
GA 12	10	3	18.5	29	15	58
GA 13	7	2	21	31.75	20.5	63.5
GA 14	12.5	3	21.5	35	21	70
GA 15	9	2.5	20	34	19	68
GA 16	16.75	3	17	23	19.25	46
GA 17	15	3	24	33	19.5	66
GA 18	13	3	22	34.5	22	69
*GA 19	12	2.5	25.5	19.5	15	39
*GA 20	14.75	3.5	25	38	24.25	76
GA 21	11	2	17.75	28	16.75	56
GA 22	14	2.5	22.5	35	20.5	70
GA 23	12	2	22	35.5	20.5	71
GA 24	11	2.5	21	32	18.5	64
GA 25	11	2.75	20	35.5	16.5	71
GA 26	12.5	2	18	30	21	60
GA 27	11.25	3.5	20.5	32.5	19	65
GA 28	12	2	19.5	32.5	21	65
GA 29	13.5	2.75	19.5	32.75	19	65.5
*GA 30	10	2	22	32.5	20.5	65

Table B1 (Continued): Physical Measurements. *Specimen removed from study.

All Measurements in Millimeters			CBD = Cannot be Determined			
Specimen	**Apical Height**	**Thickness of Test**	**Size of Ambulacra**	**Peristome–Edge**	**Periproct–Edge**	**Diameter**
GA 31	4	1	7	11	6	22
TX 1	13.75	3.2	25.5	46	CBD	92
***TX 2**	6.8	1	CBD	CBD	CBD	CBD
TX 3	10.3	1.6	CBD	CBD	CBD	CBD
TX 4	9.2	3.1	23.7	45.8	25.3	91.6
TX 5	15	3.2	22.1	43.2	25.6	86.4
TX 6	8.9	2.5	27	43.5	23.5	87
TX 7	8.8	1.3	CBD	CBD	CBD	CBD
LA 1	10	3	23.2	CBD	CBD	CBD

APPENDIX C: GIS Data

Table C1. Files used in the construction of the GIS map

File Name	Source	Description	Comments
statesarc.adf	USGS	Outlines of the United States	This file was in the preferred coordinate system for this study, GCS_Clarke_1866, and was used as the reference file for the coordinate system.
kbge.adf	USGS	Geologic map of the United States	This file shows the geologic units in the United States, was originally in a different coordinate system, and had to be transformed
USA/Conterminus/states.shp	Mgisdata	State polygon map	This file shows the outlines of the United States, were included in the textbook, Mastering ArcGIS (Price, 2008), and were used as a base for all of the additional data.
USA/usdata/cities.shp	Mgisdata	Major Cities shapefile	The cities helped pinpoint where the collection sites were located on the map
USA/usdata/transportation/interstates.shp	Mgisdata	Interstates shapefile	Interstates were also good reference points in creating the shapefile of the collection site
P.lyelli_sites.shp	Original	Collection sites	Important data from each site are connected to the point localities in the shapefile through its attribute table

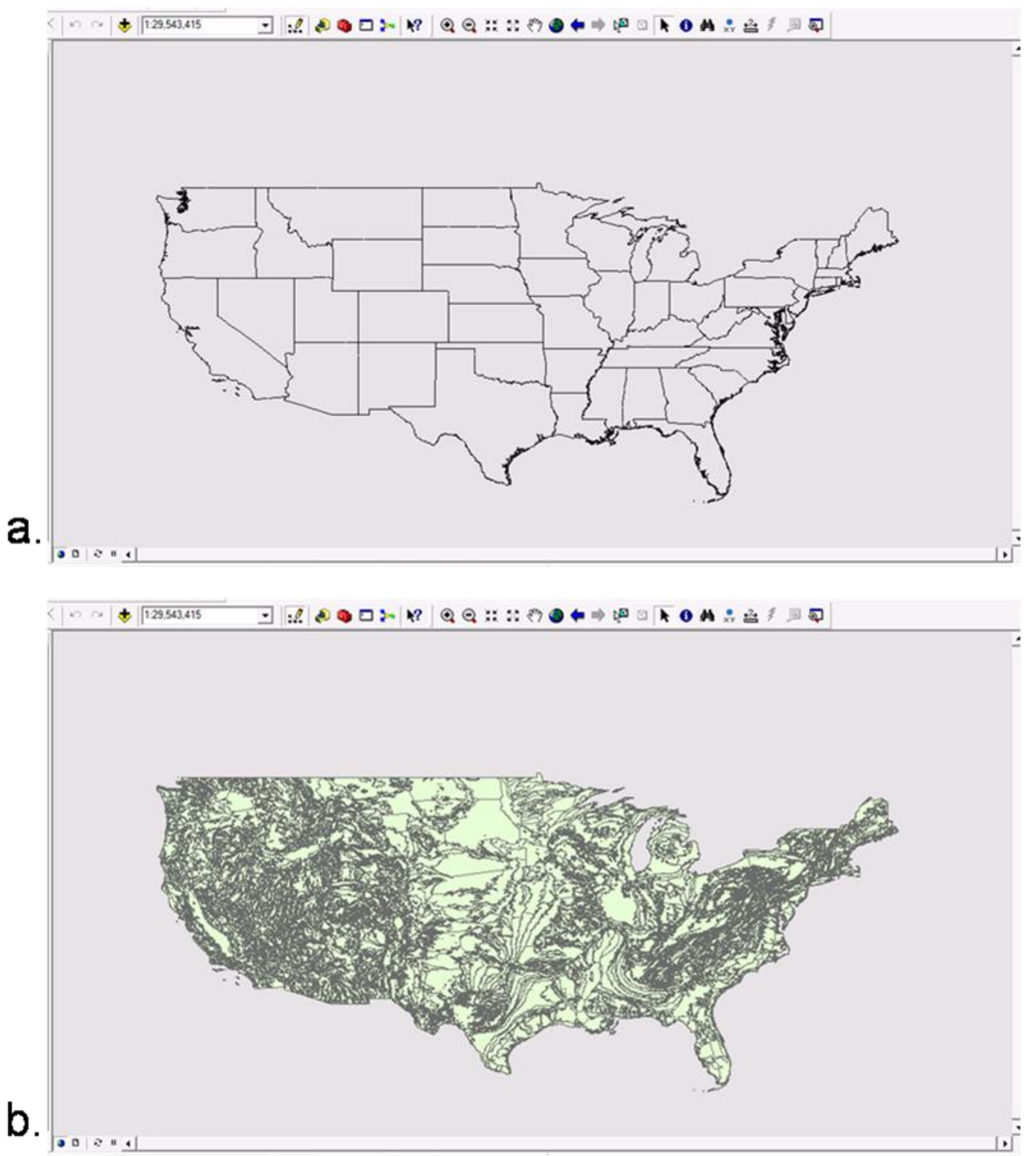


Figure 1C: Screenshots of GIS work; a. mgisdata showing state outlines; b. USGS geologic units.

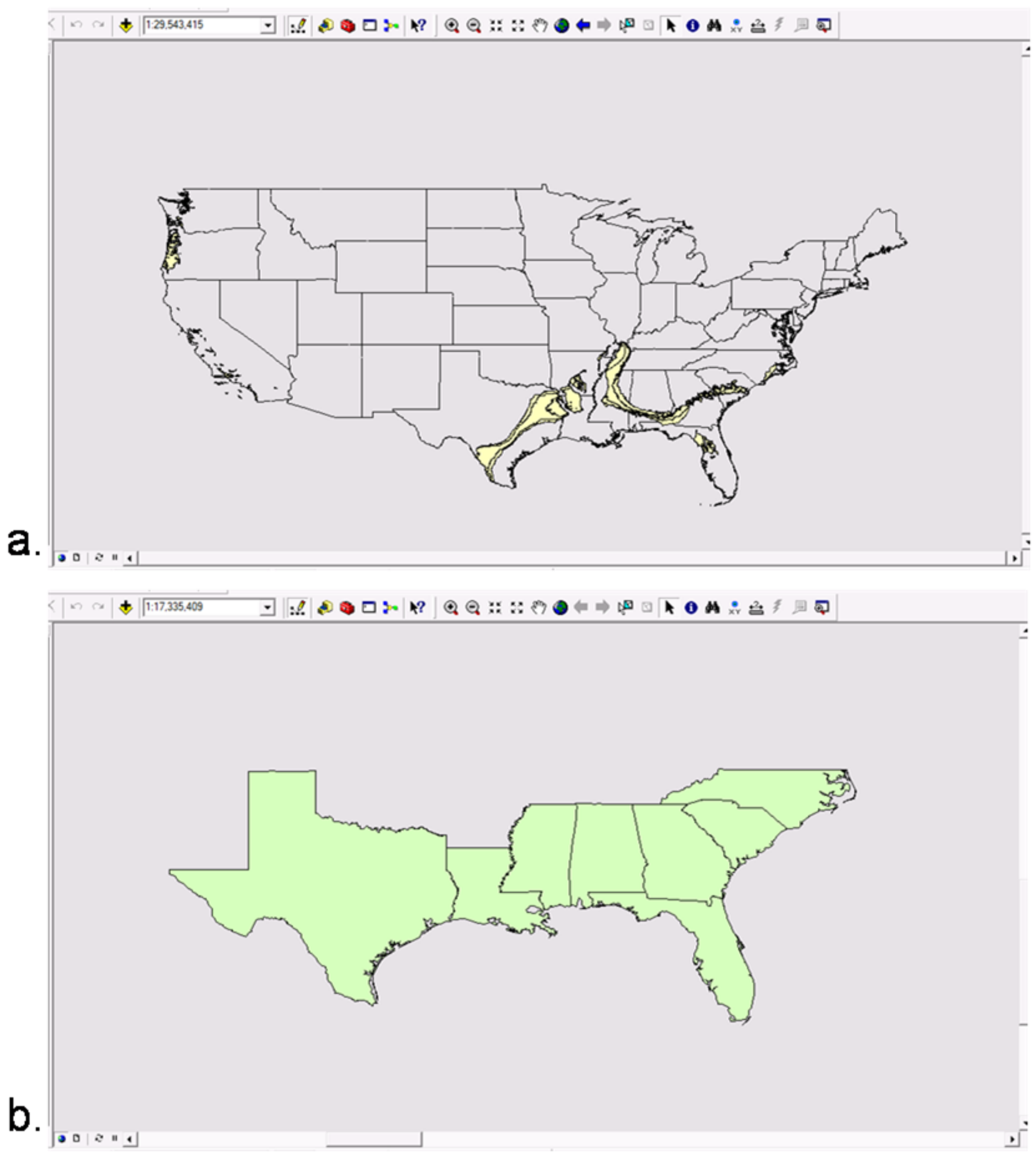
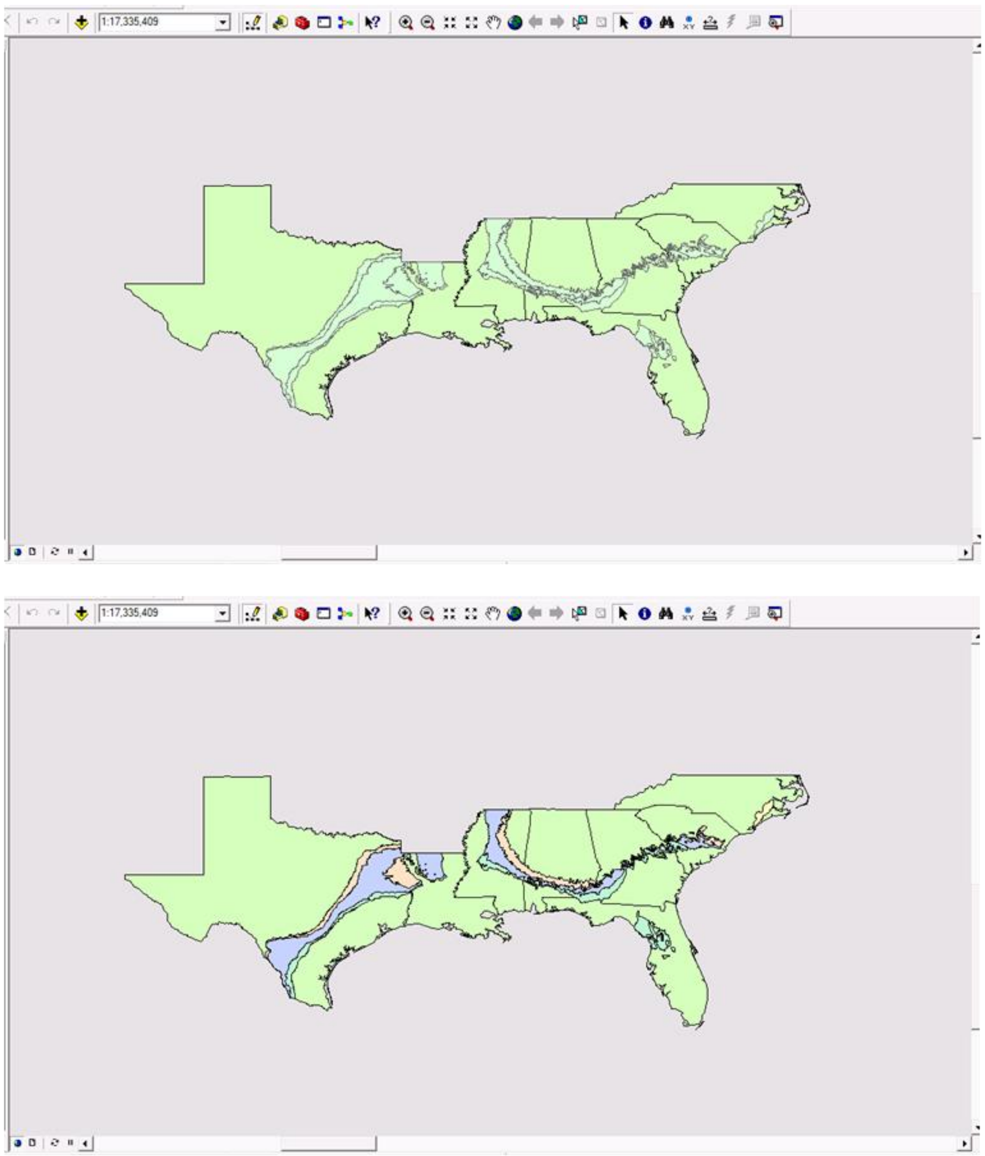


Figure 2C: Screenshots of GIS work; a. clipped data showing Eocene age geologic units; b. clipped data showing states included in study.



a.

b.

Figure 3C: Screenshots of GIS work; a. joined state and Eocene geologic unit data; b. Eocene units separated into layers according to group.

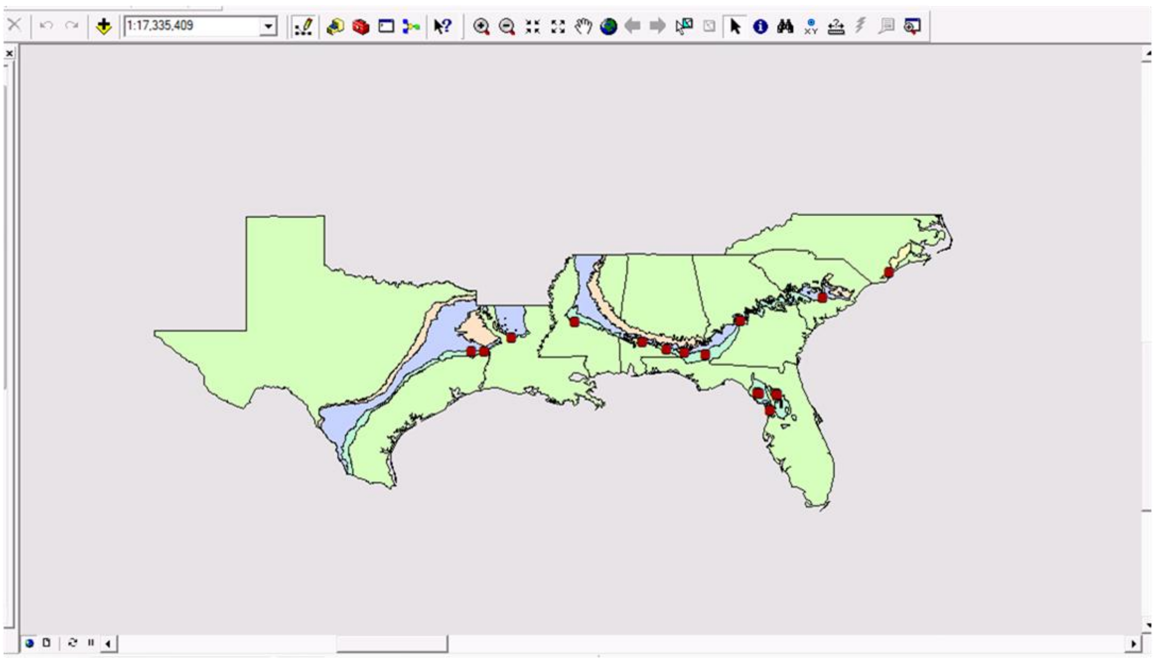


Figure 4C: GIS final product showing all layers.

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